Vol. XXVI. *June, 1914.* *No. 6*

BIOLOGICAL BULLETIN

THE MARINE BIOLOGICAL LABORATORY

SIXTEENTH REPORT

FOR THE YEAR 1913

I. LIST OF TRUSTEES 317
II. ACT OF INCORPORATION 318
III. BY-LAWS 319
IV. TREASURER'S REPORT 321
V. LIBRARIAN'S REPORT 324
VI. THE DIRECTOR'S REPORT
Introduction 326
1. The Staff—1913 331
2. Investigators and Students—1913 333
3. Tabular View of Attendance 338
4. Subscribing Institutions 339
5. Evening Lectures—1913 340
6. Members of the Corporation 340

I. TRUSTEES

EX OFFICIO

F. R. LILLIE, *Director*, The University of Chicago.
GILMAN A. DREW, *Assistant Director*, Marine Biological Laboratory.
D. BLAKELY HOAR, *Treasurer*, 161 Devonshire Street, Boston, Mass.
GARY N. CALKINS, *Clerk of the Corporation*, Columbia University.

TO SERVE UNTIL 1917

S. F. CLARKE.....Williams College.
CHARLES A. COOLIDGE...Ames Building, Boston, Mass.
C. R. CRANE.....2559 Michigan Boulevard, Chicago, Ill., President of the Board.

ALFRED G. MAYER.....Carnegie Institution.
C. E. MCCLUNG.....University of Pennsylvania.
T. H. MORGAN.....Columbia University.
ERWIN F. SMITH.....United States Department of Agriculture.
E. B. WILSON.....Columbia University.

TO SERVE UNTIL 1916

H. H. DONALDSON.....Wistar Institute of Anatomy and Biology.
M. J. GREENMAN.....Wistar Institute of Anatomy and Biology.
C. W. HARGITT.....Syracuse University.
H. S. JENNINGS.....Johns Hopkins University.
GEORGE LEFEVRE.....University of Missouri, Secretary of the Board.
A. P. MATHEWS.....University of Chicago.
G. H. PARKER.....Harvard University.

TO SERVE UNTIL 1915

H. C. BUMPUS.....University of Wisconsin.
R. A. HARPER.....Columbia University.
W. A. LOCY.....Northwestern University.
JACQUES LOEB.....The Rockefeller Institute for Medical Research.
F. P. MALL.....Johns Hopkins University.
GEORGE T. MOORE.....Missouri Botanical Garden, St. Louis.
L. L. NUNN.....Telluride, Colo.
JOHN C. PHILLIPS.....299 Berkeley Street, Boston, Mass.

TO SERVE UNTIL 1914

CORNELIA M. CLAPP....Mount Holyoke College.
E. G. CONKLIN.....Princeton University.
ROSS G. HARRISON.....Yale University.
CAMILLUS G. KIDDER....27 William Street, New York City.
M. M. METCALF.....Oberlin College.
WILLIAM PATTEN.....Dartmouth College.
JACOB REIGHARD.....University of Michigan.
W. B. SCOTT.....Princeton University.

II. ACT OF INCORPORATION

No. 3170.

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns,

Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our LORD ONE THOUSAND, EIGHT HUNDRED and EIGHTY-EIGHT.

HENRY B. PIERCE,
Secretary of the Commonwealth.

[SEAL.]

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 12 o'clock noon, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk, who shall be, *ex officio*, members of the Board of Trustees, and Trustees as hereinafter provided. At the annual meeting to be held in 1897, not more than twenty-four Trustees shall be chosen, who shall be divided into four classes, to serve one, two, three, and four years, respectively, and thereafter not more than eight Trustees shall be chosen annually for the term of four years. These officers shall hold their respective offices until others are chosen and qualified in their stead. The Director and Assistant Director, who shall be chosen by the Trustees, shall also be Trustees, *ex officio*.

II. Special meetings of the members may be called by the Trustees, to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President and may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The President shall annually appoint two Trustees, who shall constitute a committee on finance, to examine from time to time the books and accounts of the Treasurer, and to audit his accounts at the close of the year. No investments of the funds of the Corporation shall be made by the Treasurer except approved by the finance committee in writing.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be given to the Boston Society of Natural History, or some similar public institution, on such terms as may then be agreed upon.

IX. These By-Laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-Laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. TREASURER'S REPORT

Cash Receipts and Disbursements For the Year Ending December 31, 1913

Receipts

Cash on hand January 1, 1913	$ 4,788.70	
Annual dues	1,012.00	
Biological Bulletin	2,031.46	
Boats	5.00	
Carpenter shop	12.89	
Chemical department	3.17	
Charles R. Crane	21,000.00	
Donations	35.00	
Dormitory, stone building	338.25	
Dormitory, Whitman cottage	236.50	
Fish trap	337.48	
Instruction, botany	350.00	
Instruction, embryology	1,150.00	
Instruction, physiology	450.00	
Instruction, zoölogy	1,600.00	
Library	1.43	
Maintenance buildings and grounds	7.83	
Mess	12,152.26	
Mess extension	339.86	
Miscellaneous	373.24	
Pile driver	35.00	
Research	2,575.00	
Supply department	14,554.40	$63,389.47

Payments

Administration	$ 6,932.18	
Bath house	8.00	
Biological Bulletin	2,344.13	
Botany building	516.65	
Boats	4,566.01	
Carpenter shop	215.14	
Chemical department	2,022.34	

Dormitory, stone building	283.55	
Dormitory, Whitman cottage	547.10	
Fish trap	897.60	
Instruction, botany	1,125.00	
Instruction, embryology	454.31	
Instruction, physiology	650.00	
Instruction, zoölogy	1,081.00	
Lectures	48.35	
Library	1,177.41	
Maintenance buildings and grounds	4,477.27	
Mess	11,042.28	
Mess extension	3,971.12	
Miscellaneous	2,281.48	
New laboratory	740.44	
Oil house	436.86	
Philosophical lectures	100.00	
Pile driver	87.77	
Pumping station	1,890.39	
Store house	106.18	
Supply department	9,439.16	
Supply department, improvements	1,284.70	
Cash on hand January 1, 1914	4,663.05	$63,389.47

Cash Receipts and Disbursements on Account of Funds August 1, 1913, to January 1, 1914

Reserve Fund

Cash on hand August 1, 1913	$ 222.12	
Div. 6 shs. Am. Smelting & Refining Co. Pfd.	21.00	
Div. 8 shs. Gen. Elec. Co.	16.00	
Div. 14 shs. United Shoe Mach. Corp. Pfd.	5.25	
Interest on deposit	3.51	$ 267.88

Crocker Fund

Cash on hand August 1, 1913	88.79
Div. 1 sh. Am. Tel. & Tel. Co.	2.00
Div. 18 sh. Vermont & Mass. R. R. Co.	54.00

Div. 1 sh. West Wnd St. Ry. Co.	1.75	
Div. 2½ sh. Gen. Elec. Co.	5.00	
	$ 151.54	
Payment of 2 scholarships	100.00	51.54

LIBRARY FUN

Cash on hand August 1, 1913	$ 191.04	
Div. 3 shs. Am. Tel. & Tel. Co.	6.00	
Div. 1 sh. Am. Smelting & Refining Co. Pfd.	3.50	
Div. 2½ shs. Gen. Elec. Co.	5.00	
Div. 5 shs. United Shoe Mach. Corp. Pfd.	1.87	207.41
Cash on January 1, 1914		$526.83

MARINE BIOLOGICAL LABORATORY INVESTMENTS (BOOK VALUE)

JANUARY 1, 1914

RESERVE FUND

$3,000 Am. Tel. & Tel. Co. 4%	$2,921.25	
6 shs. Am. Smelting & Refining Co. Pfd.	732.00	
8 shs. Gen. Elec. Co.	972.05	
14 shs. United Shoe Mach. Corp. Pfd.	393.75	
Cash	267.88	
	$5,286.93	
Part of the above stocks and bonds is held as collateral for loan of	3,000.00	$2,286.93

LIBRARY FUND

3 shs. Am. Tel. & Tel. Co.	$ 381.18	
4/5 of $1,000 Am. Tel. & Tel. Co. 4%	779.00	
1 sh. Am. Smelting & Refining Co. Pfd.	122.00	
2½ shs. Gen. Elec. Co.	288.10	
5 shs. United Shoe Mach. Corp. Pfd.	140.63	
Cash	207.41	1,918.32

LUCRETIA CROCKER FUND

18 shs. Vermont & Mass. R. R. Co.	$2,416.50
1 sh. West End St. Ry. Co.	83.00
1 sh. Am. Tel. & Tel. Co.	127.06

1/5 of $1,000 Am. Tel. & Tel. Co. 4%	194.75	
2½ shs. Gen. Elec. Co.	323.85	
Cash	51.54	3,196.70
		$7,401.95

V. LIBRARIAN'S REPORT

AUGUST, 1913

With expenditures about the same, approximately $1,000, the library has been considerably improved in several ways since last year.

1. Most important is the gift from the Carnegie Institution of a complete set of publications relating to our work. There are very few laboratories in this list, so the placing of the library on the mailing list is a decided gain. In this way about 85 valuable monographs, not otherwise available, have been received.

2. Last year, about this time, a strong representation was placed before a number of publishing firms, of the advantages to them of sending here copies of works on science and education. The response was very prompt and generous; and as a result about 150 books have been added representing a value of over $250. A word to publishers on the part of authors will now be sufficient to explain to them the value of such advertisement as is furnished by a place on our shelves.

3. In response to letters enclosing lists showing missing parts and asking aid in completing our sets, we have been able to fill in some bad gaps; and are still receiving assistance here. The lists are available to anyone who will help.

4. An important departure, which we hope will establish a precedent, has been the gift of subscriptions to new journals by members of the corporation: *The Popular Science Monthly*, *The American Journal of Anatomy*, *The American Naturalist*, *The Annual Bulletin of the Zoölogical Museum of the Imp. Acad. of Science, St. Petersburg*, and *The Journal of The Society for Experimental Biology and Medicine*. Drs. A. Mayer, Bumpus, and Knower have each given $10 a year for five years toward journals, either for new subscriptions or missing parts of back sets. Much

extension of this assistance is desirable as more journals are greatly needed and we are only spending at the present time about $700 for this purpose.

5. We have added by exchange: *Bulletin Scientifique de La France et Belgique*, Vols. 26 to 46; *Mittheilungen aus dem Naturhistorisches Museum in Hamburg*, Vols. 7 to 29; also a complete series of the *Monist* and the *Wilson Bulletin* from Oberlin, O.

6. The reprint collection has been greatly improved by rearrangement and considerable addition. We must, however, appeal again for gifts to this collection. It is in much demand and we are quite dependent on authors to remember this.

Finally, the librarian feels obliged to urge the great importance of now engaging a trained assistant with ability to carry on, throughout the year, a systematic campaign to develop the library. This work should be done under the general direction of the librarian, by maintaining a business-like correspondence in regard to accessions, exchanges, missing parts, etc. It is the only way in which we can steadily and adequately improve. The peculiarly free methods used in this library demand constant study of the shelves, catalogues, etc., to take account of losses, needed repairs and disarrangements caused by the free access to the shelves. This is especially necessary after the summer session; otherwise the entire collection becomes more or less disorganized and chaotic and there is inevitable loss from year to year. The binding, catalogues, special sets, repairs, reprints have to be constantly watched and kept strictly up to date, to make the material which we have more available. We have been suffering from very inadequate and irregular assistance.

Dr. Drew's work on the catalogues and binding and the enterprise of his office in pushing some of the schemes which have improved things have demonstrated what can and should be done.

This summer, as soon as Dr. Drew's office force was diverted into its regular work, we were again obliged to drop back to carrying through the routine demands of the busy season, with what aid we could secure. Much more could and should be done through a permanent assistant librarian keeping behind the library work winter and summer.

I should recommend that such an appointment be made in advance of the move to our permenent quarters; since this should be supervised by an experienced person, and because there is important preliminary work now awaiting attention.

H. Mc. E. KNOWER,
Librarian.

VI. THE DIRECTOR'S REPORT

TO THE TRUSTEES OF THE MARINE BIOLOGICAL LABORATORY:

Gentlemen: I have the honor to transmit herewith a report of the twenty-sixth session of the Marine Biological Laboratory. The year just drawing to a close has been in many respects the most successful and encouraging since the foundation of the Laboratory. Not only has there been a great increase in attendance which has tasked our accommodations beyond our previous estimates of the uttermost, but the spirit of research has never been more intense or on a higher plane, and many interesting results have been obtained. If these could in some way be exhibited together I feel confident that they would amount to a really great increase in the resources of biological facts and principles. This matter is, however, intangible and impossible to estimate at close range. Even after publication the results of a particular piece of investigation are not capable of immediate appraisal; and but little of last season's work is yet published. Moreover in our absolutely free method of organization with no prescription as to material, or subject matter, or method of investigation, and no restriction as to time or place of publication, one can gain only the most general impression of the results actually secured by so large a body of investigators. I can only feel, and express the opinion, that the laboratory is maintaining its best traditions in these respects.

Turning to the record of the principal events of the year: the attendance of the investigators was in excess of the largest previous attendance by nearly one third; the actual figures are 122 in 1913, 93 in 1912, 82 in 1911, 62 in 1910. The number of students in courses was 69, making a total attendance of in-

vestigators and students of 191, as compared with 160 in 1912, 147 in 1911 and 126 in 1910. The number of subscribing institutions was 30 in 1913, as compared with 29 in 1912, 25 in 1911, 24 in 1910, 20 in 1909, 18 in 1908 and 16 in 1907. The list is given on p. 339. Of these Barnard College, the Crocker Research Fund of Columbia University, Harvard University, Kansas State Agricultural College and Radcliffe College are new subscriptions. The receipts from the subscribing institutions and students' fees were $6,160 in 1913, as compared with $5,175 in 1912, $4,574.99 in 1911, $4,150 in 1910 and $3,700.35 in 1909. The receipts from the supply department were $14,554.40 in 1913, as compared with $13,966.35 in 1912, $10,303.61 in 1911, $9,300.58 in 1910, and $8,549.55 in 1909.

At the beginning of 1913 a new system of book-keeping was established with the aid of Dr. Drew. While this may cause some difficulty in comparison of the treasurer's report for the current year with the preceding reports, it gives a much more accurate idea of the real classification of expenses and the cost of departments, and it is hoped that it may furnish the basis for more complete comparisons in the future. The book-keeping is now done at Wood's Hole, under the supervision of the treasurer, instead of in Boston as formerly; this is a great aid in administration, as it enables the executive officers to keep closer account of receipts and expenditures.

Great improvements were made in the mess in preparation for the past summer session. The dining-room was enlarged by about 50 per cent., a wide covered verandah was built around three sides of it; the kitchen was much enlarged and its ventilation improved and a new laundry built. The expense of these improvements, $5,720.16, was provided for partly by the earnings of the mess $1,581.70, partly by subscriptions amounting to $2,147.33 from a considerable number of subscribers, and partly by current funds of the Laboratory to which the mess now stands indebted in the sum of $1,991.13.

The large increase of investigators was accommodated by utilizing the Kidder Annex, the Yacht Club building, and every available corner of the old buildings. The progress of the new building was therefore particularly inspiriting in its promise of

relief from overcrowding. We purpose to publish an illustrated account of this building in the BIOLOGICAL BULLETIN at an early date. We shall therefore deal briefly with it here:

At the winter meeting of the board of trustees in 1909 a committee was appointed to prepare plans for a permanent building of fireproof construction, to accommodate the library and to include a number of research laboratories equipped for more delicate researches and capable of being used at all seasons of the year. The president of the board, Mr. Charles R. Crane, promised to contribute the necessary funds for construction, and our fellow member of the board, Mr. Charles A. Coolidge, the distinguished architect, agreed to furnish the plans of the building as his contribution. Such concurrence of generous support has rarely happened in the history of any institution. Plans were carefully studied by the committee in charge, and an interior arrangement best suited to our needs was agreed on; Mr. Coolidge then prepared various exterior plans. After much study and discussion it was finally agreed that the simplest plan of architecture was most in keeping with our traditions and the location. In the summer of 1912 the plans were complete. They were submitted to many of the workers at the laboratory for criticism and suggestion and were finally approved by the board. The contracts were signed that winter and construction was begun in February, 1913.

The location decided on was selected as part of a plan for the location of several more buildings of permanent construction. The new building faces south on the Woods Hole Harbor. It is constructed of tapestry brick with stone trimmings; in form rectangular 92 by 50 feet, three stories and a high basement. The height of the three stories, the proportions of the openings, the construction of the cornice, and the nature of the materials combine to produce a pleasing effect of great dignity. The building is now nearly completed and will be ready for occupancy in March, 1914.

Hitherto the work of the Laboratory has been carried on in buildings of an avowedly temporary character. The completion of the first permanent building, therefore, marks a stage in the growth of the laboratory deserving of especial recognition. I,

therefore, recommend to the board, at the suggestion of the president, that special exercises of dedication be held to commemorate the event early in the next summer session, and that a committee be appointed to make arrangements therefor. The significance of the event relates not only to the development of our institution, but also especially to the dedication of so adequate an equipment for the purposes of research in pure science outside of our universities.

Among the clearly defined tendencies in the growth of our institution is the increase in numbers of our membership. As I pointed out in my last report the living accommodations in Woods Hole for transient workers at the Laboratory are actually inadequate. We need more dormitory space, especially for women. The suggestion in my last report that one of the smaller laboratory buildings might be made over as a dormitory for women seems now to be impracticable, if the increase in attendance of last summer is any indication of what to expect in the future.

There is a great need of cottages for the families of workers at the Laboratory. Those available are held at exorbitant rents. As the personnel of the investigators is really our prime consideration this is a problem of importance. By coöperation it should be possible for such persons to build bungalows without financial loss. But unless some plan is organized from the Laboratory nothing is likely to be done, because the people interested are not in touch with one another. It would be an important aid to the cause of science if someone would erect a group of small cottages that might be rented for a small return on the investment at from $100 to $200 for the season.

We have provided a fine library room well equipped for several times the present number of volumes. The development of the library should now be made a special problem. The appointment of a library assistant for the entire year would be a most desirable step in this direction. Until this is done, the development of the library is likely to be spasmodic and slower than need be. I refer to the report of the librarian (p. 324) on this subject.

A new and larger lecture hall is another immediate need and the improvement of the rooming house for employees of the mess yet another.

The equipment of the new laboratory has been provided for by an additional gift from Mr. Crane, and the executive officers have proceeded with the necessary plans in order to avoid delay in the opening of the building. The most important part of the preparation, viz: provision for adequate pumping facilities, is completely arranged for. It involves construction of a wharf on the harbor front to carry a supply pipe out to deep water, but also useful for other laboratory purposes, and the installation of pumps and the power plant. After considerable inquiry we decided on hard rubber pumps of such construction that no metal can come in contact with the sea-water. We believe that all sources of metal contamination of the sea-water in the system of pumping and distribution have been avoided. The valves in the system are of special lead, manufactured by the Crane Valve Company, thus avoiding a source of metal contamination in the use of brass or bronze valves. The power will be furnished by electric motors with a reserve gasoline engine. Other details of equipment need not be included in this report.

At the last meeting of the board of trustees the director and assistant director were authorized to proceed with plans for the improvement of the water front. Surveys were accordingly made, and plans prepared for the erection of sea-walls both on the harbor and eel-pond frontage; wharves in connection with these improvement were also planned, and filling and grading in order to utilize the space in the best possible way. Such part of these plans as required state permits were presented to the harbor and land commission of the state of Massachusetts and the permits secured. The drawings of these projects are herewith presented.

It is not necessary to proceed with all of this work at once; but it is essential that anything undertaken should be part of a general plan, and this is the idea we have had in mind in planning so much at once.

We still have before us the necessity of an endowment before we can feel certain that the operation of the Laboratory can be continued uninterruptedly. The estimates for the year 1914 show a probable deficit of about $20,000, a very small sum considering the magnitude and significance of our operations. An

endowment of $500,000 would permanently insure our present status at least, and I feel very strongly that every effort should be made to raise such a sum before we prepare for farther expansion.

I. THE STAFF

1913

F. R. LILLIE, DIRECTOR,
Professor of Embryology, The University of Chicago.

GILMAN A. DREW, ASSISTANT DIRECTOR,
Marine Biological Laboratory.

ZOÖLOGY

I. INVESTIGATION

Zoölogy and Embryology

GARY N. CALKINS.....Professor of Protozoölogy, Columbia University.
E. G. CONKLIN.....Professor of Zoölogy, Princeton University.
GILMAN A. DREW.....Assistant Director, Marine Biological Laboratory.
GEORGE LEFEVRE.....Professor of Zoölogy, University of Missouri.
FRANK R. LILLIE.....Professor of Embryology, The University of Chicago.
T. H. MORGAN.....Professor of Experimental Zoölogy, Columbia University.
E. B. WILSON.....Professor of Zoölogy, Columbia University.

II. INSTRUCTION

CASWELL GRAVE.....Associate Professor of Zoölogy, Johns Hopkins University.
GEORGE A. BAITSELL....Dean and Professor of Biology, Central College.
RAYMOND BINFORD.....Professor of Biology, Guilford College.
J. K. BREITENBECKER ...Fellow in Zoölogy, University of Chicago.
E. J. LUND.....Bruce Fellow in Zoölogy, Johns Hopkins University.
T. S. PAINTER.....Graduate Student of Zoölogy, Yale University.

EMBRYOLOGY

I. INVESTIGATION (See Zoology)

II. INSTRUCTION

GILMAN A. DREW.....Assistant Director, Marine Biological Laboratory.

LORANDE L. WOODRUFF..Assistant Professor of Biology, Yale University.

A. L. TREADWELL.....Professor of Biology, Vassar College.

ROBERT A. BUDINGTON...Associate Professor of Zoölogy, Oberlin College.

PHYSIOLOGY

I. INVESTIGATION

ALBERT P. MATHEWS....Professor of Physiological Chemistry, The University of Chicago.

R. S. LILLIE.....Assistant Professor of Experimental Biology, University of Pennsylvania.

HAROLD C. BRADLEY.....Assistant Professor of Physiological Chemistry, University of Wisconsin.

II. INSTRUCTION

RALPH S. LILLIE.....Assistant Professor of Experimental Biology, University of Pennsylvania.

WALTER E. GARREY.....Associate Professor of Physiology, Washington University Medical School.

FRANK P. KNOWLTON...Professor of Physiology, Syracuse University.

EDWARD B. MEIGS.....Associate in Physiology, Wistar Institute of Anatomy and Biology.

PHILOSOPHICAL ASPECTS OF BIOLOGY AND ALLIED SCIENCES

LECTURES

EDWARD G. SPAULDING...Assistant Professor of Philosophy, Princeton University.

BOTANY

GEORGE T. MOORE.....Engelmann Professor of Botany, Washington University.

GEORGE R. LYMAN.....Assistant Professor of Botany, Dartmouth College.

B. M. DUGGAR.....Professor of Plant Physiology, Washington University.

IVEY F. LEWIS.....Assistant Professor of Botany, University of Wisconsin.
W. J. ROBBINS.....Assistant in Plant Physiology, Cornell University.
R. H. COLLEY.....Instructor in Botany, Dartmouth College.
A. R. DAVIS.....Lockland Research Fellow, Shaw School of Botany.

LIBRARY

H. McE. KNOWER.....University of Cincinnati, Librarian.

CHEMICAL SUPPLIES

OLIVER S. STRONG.....College of Physicians and Surgeons, New York City, Chemist.

G. M. GRAY.....Curator of Supply Department.
A. L. LEATHERS.....Collector.
JOHN VEEDER.....Cockswain.
F. M. MACNAUGHT.....Business Assistant.

2. INVESTIGATORS AND STUDENTS

1913

INVESTIGATORS 1913

ZOÖLOGY

Independent Investigators

ALLEE, W. C., Instructor in Zoölogy, Williams College.
BAITSELL, GEORGE A., Graduate Student, Yale University.
BECKWITH, CORA J., Instructor in Biology, Vassar College.
BINFORD, RAYMOND, Professor of Biology, Guilford College.
BORING, ALICE M., Associate Professor of Zoölogy, University of Maine.
BREITENBECKER, J. K., Instructor in Biology, Western Reserve University.
BROWNE, ETHEL N., Instructor in Biology, Dana Hall, Wellesley College.
BUDINGTON, ROBERT A., Associate Professor of Zoölogy, Oberlin College.
BULLOCK, F. D., Associate in Cancer Research, Columbia University.
CALKINS, GARY N., Professor of Protozoölogy, Columbia University.
CHAMBERS, ROBERT, Assistant Professor of Histology and Comparative Anatomy, University of Cincinnati.
CHILD, C. M., Associate Professor of Zoölogy, University of Chicago.

CLAPP, CORNELIA M., Professor of Zoölogy, Mount Holyoke College.
CONKLIN, E. G., Professor of Biology, Princeton University.
CRAMPTON, H. E., Professor of Zoölogy, Barnard College, Columbia University.
DREW, GILMAN A., Assistant Director, Marine Biological Laboratory.
EDWARDS, DAYTON J., Tutor in Physiology, College of the City of New York.
GLASER, O. C., Junior Professor of Zoölogy, University of Michigan.
GOLDFARB, A. J., Instructor in Zoölogy, College of the City of New York.
GRAVE, CASWELL, Professor of Zoölogy, Johns Hopkins University.
GRAVE, B. H., Professor of Biology, Knox College, Galesburg, Ill.
GREGORY, LOUISE H., Instructor in Zoölogy, Barnard College.
HARVEY, E. N., Instructor in Physiology, Princeton University.
HEGNER, R. W., Assistant Professor of Zoölogy, University of Michigan.
HOGUE, MARY J., Instructor in Zoölogy, Mount Holyoke College.
HYDE, R. R., Assistant Professor of Physiology and Zoölogy, Indiana State Normal School.
JACKSON, ROBERT T., Professor of Paleontology, Harvard University.
JUST, E. E., Professor of Zoölogy, Howard University.
KNOWER, H. McE., Professor of Anatomy, University of Cincinnati.
LEFEVRE, GEORGE, Professor of Zoölogy, University of Missouri.
LILLIE, FRANK R., Professor of Embryology, University of Chicago.
LUND, E. J., Adam T. Bruce Fellow, Johns Hopkins University.
MCCLUNG, C. E., Professor of Zoölogy, University of Pennsylvania.
MCGREGOR, J. H., Professor of Zoölogy, Columbia University.
MALL, F. P., Professor of Anatomy, Johns Hopkins University.
MALONE, E. F., Assistant Professor of Anatomy, University of Cincinnati.
MORGAN, T. H., Professor of Experimental Zoölogy, Columbia University.
MORRILL, C. V., Instructor in Anatomy, New York University.
MORSE, EDWARD S., Director, Peabody Museum, Salem, Mass.
NEWMAN, H. H., Associate Professor of Zoölogy, University of Chicago.
PAINTER, T. S., Instructor in Zoölogy, Roanoke College.
PAPPENHEIMER, A. M., Associate in Pathology, Columbia University.
PARMENTER, C. S., Vice-president and Professor of Zoölogy, Baker University, Baldwin, Kansas.
PATON, STEWART, Lecturer in Biology, Princeton University.
PATTERSON, J. T., Professor of Zoölogy, University of Texas.
REINKE, E. E., Fellow in Zoölogy, Princeton University.
ROBERTSON, W. R. B., Assistant Professor of Zoölogy, University of Kansas.
SHOREY, MARIAN L., Professor of Biology, Milwaukee-Downer College.
SHULL, A. FRANKLIN, Assistant Professor of Zoölogy, University of Michigan.
SPAETH, R. A., Research Student, Harvard University.
SPAULDING, E. G., Assistant Professor of Philosophy, Princeton University.
STOCKARD, C. R., Professor of Anatomy, Cornell Medical College
STRONG, O. S., Instructor in Anatomy, Columbia University.
STRONG, R. M., Instructor in Zoölogy, University of Chicago.
THOMPSON, CAROLINE B., Associate Professor of Zoölogy, Wellesley College.
TREADWELL, A. L., Professor of Biology, Vassar College.
VAN CLEAVE, H. N., Instructor in Zoölogy, University of Illinois.
WILSON, E. B., Professor of Zoölogy, Columbia University.
WOODRUFF, L. L., Assistant Professor of Biology, Yale University.

Beginning Investigators

BRIDGES, CALVIN B., Graduate Student, Columbia University.
CARVER, GAIL L., Professor of Biology, Mercer University.
DEXTER, JOHN S., Fellow in Zoölogy, Columbia University.
FAUST, E. C., Research Assistant, University of Illinois.
FISH, J. BURTON, Graduate Student, Columbia University.
GLASER, R. W., Bussey Institution, Forest Hills, Boston, Mass.
GOODRICH, H. B., Assistant in Zoölogy, Columbia University.
HAYDEN, MARGARET A., Instructor in Biology, Carnegie Institute of Technology.
HEILBRUNN, L. V., Laboratory Assistant in Zoölogy, University of Chicago.
HOGE, MILDRED A., Graduate Student, Columbia University.
ISAACS, RAPHAEL, Assistant in Zoölogy and Embryology, University of Cincinnati.
LINKINS, R. H., Assistant in Zoölogy, University of Illinois.
LYNCH, CLARA J., Instructor in Zoölogy, Smith College.
MACDOWELL, E. C., Graduate Student, Harvard University.
MORRIS, MARGARET, 53 Edgehill Road, New Haven, Conn.
PACKARD, CHARLES, Assistant in Zoölogy, Columbia University.
SHUMWAY, WALDO, University Scholar in Zoölogy, Columbia University.
STARK, MARY B., Graduate Student, Columbia University.
STURTEVANT, A. H., Graduate Student, Columbia University.
WARDWELL, E. H., Assistant in Biology, Princeton University.
WHEELER, ISABEL, 18 The Hattersley, Toledo, Ohio.

PHYSIOLOGY

Independent Investigators

BANCROFT, F. W., Associate Member in Department of Experimental Biology, Rockefeller Institute for Medical Research.
BRADLEY, H. C., Assistant Professor of Physiological Chemistry, University of Wisconsin.
DONALDSON, H. H., Wistar Institute of Anatomy and Biology.
EWALD, W. F., Fellow, Rockefeller Institute for Medical Research.
GARREY, W. E., Associate Professor of Physiology, Washington University.
HYDE, IDA H., Professor of Physiology, University of Kansas.
KITE, G. L., Assistant in Physiological Chemistry, University of Chicago.
KNOWLTON, F. P., Professor of Physiology, Syracuse University.
LILLIE, R. S., Assistant Professor of Experimental Zoölogy, University of Pennsylvania.
LOEB, JACQUES, Head of Department of Experimental Biology, Rockefeller Institute for Medical Research.
MATHEWS, A. P., Professor of Physiological Chemistry, University of Chicago.
MEIGS, E. B., Wistar Institute of Anatomy and Biology.
MOORE, A. H., Associate Professor of Physiology, Bryn Mawr College.
MORSE, MAX W., Trinity College, Hartford, Conn.
TASHIRO, SHIRO, Associate in Physiology, University of Chicago.
WASTENEYS, HARDOLPH, Associate in Experimental Biology, Rockefeller Institute for Medical Research.
WHERRY, W. B., Associate Professor of Bacteriology, University of Cincinna

Beginning Investigators

ADAMS, H. S., Fellow in Chemistry, University of Chicago.
CATTELL, MCKEEN, Student, Columbia University.
GOULD, H. N., Fellow in Biology, Princeton University.
KANDA, SAKYO, Fellow in Psychology, Clark University.
LLOYD, DOROTHY J., 16 Ampton Road, Edghaston, Birmingham, England.
OLIVER, WADE W., Graduate Student, University of Cincinnati.
STRINGER, CAROLINE E., Head of Biology Department, Omaha High School.

BOTANY

Independent

DUGGAR, B. M., Research Professor of Plant Physiology, Washington University.
GARBER, JOHN F., Head of Botany Department, Yeatman High School, St. Louis, Mo.
HIBBARD, RUFUS P., Instructor in Plant Physiology, Michigan Agricultural College.
LEWIS, I. F., Assistant Professor of Botany, University of Wisconsin.
LYMAN, GEORGE R., Assistant Professor of Botany, Dartmouth College.
MOORE, GEORGE T., Director, Missouri Botanical Gardens.
NICHOLS, SUSAN P., Associate Professor of Botany, Oberlin College.
OSTERHOUT, W. J. V., Professor of Botany, Harvard University.
SNOW, LAETITIA M., Associate Professor of Botany, Wellesley College.
STOMPS, THEODOR J., Professor of Cytology, University of Amsterdam.
WUIST, ELIZABETH D., 2351 East 5th Street, Dayton, Ohio.

Beginning Investigators

COLLEY, R. H., Instructor in Biology, Dartmouth College.
CURTIS, OTIS F., Instructor in Botany, Cornell University.
DAVIS, A. R., Lackland Research Fellow, Washington University.
FOSTER, GOODWIN L., Graduate Student, Dartmouth College.
HOPPING, ALEITA, Tottenville, Staten Island, New York.
ROBBINS, W. J., Instructor in Plant Physiology, Cornell University.
ROBERTS, EDITH A., Instructor in Botany, Mount Holyoke College.

STUDENTS

1913

ZOÖLOGY

ABBOTT, CHARLES H., Student, Brown University.
ALLEN, CHARLES E., Student, Wabash College.
ATWOOD, WARREN G., Student, Dartmouth College.
BAKER, FLORENCE I., Student, Carleton College.
BAUMANN, EMIL J., Student, Yale University.
BEYER, HENRY G., U. S. Navy, Retired.
BOBB, THOMAS N., Instructor in Zoölogy, Northland College.
BRADLEY, BARBARA, Technical Assistant in Zoölogy, University of Wisconsin.

CASHMANN, MARGUERITE, Student, Syracuse University.
CLAPP, ISABEL C., Student, Oberlin College.
DALEY, MARY W., Teacher of Physics, Dana Hall, Wellesley College.
EWALD, MARINA, 78 Kaiserin Augusta Str., Berlin N. 10, Germany.
HARP, MARGERY B., Student, Syracuse University.
HEAPS, PEARL I., Head of Biology Department, Western High School, Baltimore. Md.
HOAR, CARL S., Austin Teaching Fellow, Harvard University.
KEITH, GERALD, Student, Amherst College.
LEWIS, ELSIE M., Student, Oberlin College.
LINTON, EDWIN S., Student, Washington and Jefferson College.
MAHNKEN, FLORENCE V., 598 E. 167th Street, New York City.
MCFARLAND, HELEN J., Student, Bryn Mawr College.
MALLARD, AGNES K., Teacher, Boston Elementary School.
NELLIGAN, KATHERINE, Student, Mount Holyoke College.
PATTEN, MARY W., Student, Goucher College.
PINKERTON, MARY B., 408 Warren Crescent, Norfolk, Va.
PLOUGH, HAROLD H., Laboratory Assistant, Amherst College.
RIEKE, BENJAMIN W., Student, Carleton College.
ROBERTS, AMABEL S., Madison, N. J.
SCHULTE, ANNE M., Student, Sweet Briar College.
STONE, PHEBE, Student, Goucher College.
WARE, CLARA C., Hingham, Mass.
WILSON, CHARLOTTA W., Technical Assistant, Johns Hopkins University.
WOGLOM, WILLIAM H., Assistant Professor, Assigned to Cancer Research, Columbia University.

EMBRYOLOGY

ALLEN, EZRA, Professor of Biology, School of Pedagogy, Philadelphia.
ALLEN, GEORGE D., Instructor in Zoölogy, University of Minnesota.
BEERS, CATHERINE V., Student, Northwestern University.
BEHRE, ELINOR H., Instructor in Zoölogy, Sophie Newcomb College, Tulane University.
BURR, HAROLD S., Currier Fellow, Sheffield Scientific School, Yale University.
COOK, DELLAR L., 28 Andrews Street, Woonsocket, R. I.
FARNAM, LOUISE W., 43 Hillhouse Avenue, New Haven, Conn.
FIELD, HAZEL E., Head of Science Department, Belhaven College, Jackson, Miss.
GARDNER, KATHERINE, Student, Mount Holyoke College.
GUNTHER, MAUDE C., Instructor in Biology, Eastern High School, Washington, D. C.
HANCE, ROBERT T., Assistant in Biology, University of Cincinnati.
KNIGHT, MARIAN V., Demonstrator in Zoölogy, Smith College.
LEE, BARBARA M., Teacher of Physics and Chemistry, High School, Puyallup, Wash.
LOWTHER, FLORENCE DEL., Graduate Student, Barnard College.
MCMORLAND, EDWARD E., Assistant in Zoölogy, University of Missouri.
MARTIN, BERTHA E., Graduate Student, University of Chicago.
MAY, H. G., Graduate Student, University of Rochester.
MEIERHOF, HAROLD L., Student, Columbia University.
PHIPPS, CHARLES F., Instructor, School of Education, University of Chicago.

RUDDIMAN, MARGUERITE, 441 Senator Street, Brooklyn, New York.
TURNER, CLARENCE L., Ohio Wesleyan University, Delaware, Ohio.
YOCUM, HARRY B., Instructor, Wabash College.
YOUNG, DONNELL B., Laboratory Instructor, Amherst College.

PHYSIOLOGY

ANGLE, BROWNIE, 714 Washington Blvd., Kansas City, Kansas.
DUFF, DOROTHY, 49 Crescent Street, Montreal, Canada.
KORTRIGHT, W. H., Student, Syracuse University.
MATEER, JOHN G., Student, Wooster University.
OLMSTED, J. M. D., Assistant Professor of Biology, Richmond College.
PHILLIPS, RUTH L., Instructor in Biology, Western College.
ROOT, FRANCIS M., Graduate Student, Johns Hopkins University.
TRUEBLOOD, IRA C., Head of Biological Department, High School, Greencastle, Indiana.

BOTANY

FINK, HENRI, St. Mathew's, Jefferson County, Kentucky.
JOHNSTON, SARAH, 18 Franklin Street, Northampton, Mass.
THOMPSON, BERTHA E., Instructor, Michigan Agricultural College.
THURLOW, MADGE D., Goucher College.
SMITH, EDITH L., Assistant, West Roxbury High School.
STEWART, MARY W., Assistant, Barnard College, Columbia University.

3. TABULAR VIEW OF ATTENDANCE

	1910	1911	1912	1913
INVESTIGATORS—Total	62	82	93	122
Independent				
Zoölogy	33	42	44	58
Physiology	11	18	14	17
Botany	9	8	10	11
Under Instruction				
Zoölogy	6	12	21	21
Physiology	2	2	2	7
Botany			2	7
STUDENTS—Total	64	65	67	69
Zoölogy	31	26	24	33
Embryology	10	20	15	22
Physiology	5	6	11	8
Botany	17	13	17	7
TOTAL ATTENDANCE	126	147	160	191

INSTITUTIONS REPRESENTED				
Total			57	80
By investigators	26	37	43	50
By students	24	31	36	41
SCHOOLS AND ACADEMIES REPRESENTED				
By investigators	5	3	2	3
By students	6	9	1	6

4. SUBSCRIBING INSTITUTIONS, 1913

BARNARD COLLEGE.
BRYN MAWR COLLEGE.
CARLETON COLLEGE.
COLUMBIA UNIVERSITY.
CROCKER RESEARCH FUND OF COLUMBIA UNIVERSITY.
DARTMOUTH COLLEGE.
ELSE SERINGHAUS SCHOLARSHIP OF NORMAL COLLEGE.
GOUCHER COLLEGE.
HARVARD UNIVERSITY.
KANSAS STATE AGRICULTURAL COLLEGE.
LUCRETIA CROCKER SCHOLARSHIPS.
MOUNT HOLYOKE COLLEGE.
NORTHWESTERN UNIVERSITY.
OBERLIN COLLEGE.
PRINCETON UNIVERSITY.
RADCLIFFE COLLEGE.
ROCHESTER UNIVERSITY.
ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH.
SMITH COLLEGE.
SYRACUSE UNIVERSITY.
UNIVERSITY OF CHICAGO.
UNIVERSITY OF CINCINNATI.
UNIVERSITY OF ILLINOIS.
UNIVERSITY OF MICHIGAN.
UNIVERSITY OF MISSOURI.
UNIVERSITY OF PENNSYLVANIA.
VASSAR COLLEGE.
WELLESLEY COLLEGE.
WISTAR INSTITUTE OF ANATOMY AND BIOLOGY.
YALE UNIVERSITY.

5. EVENING LECTURES, 1913

H. E. CRAMPTON....."Four Journeys among the Islands of Polynesia".....July 3.
A. G. MAYER....."Effects of the Electrolytes of Sea-water upon the Rate of Nerve Conduction in *Cassiopea*".....July 8.
JOHN AUER....."Anaphylaxis".....July 11.
T. H. MORGAN....."Heredity in *Drosophila*".....July 15.
STEWART PATON....."The National Campaign for Mental Hygiene in Relation to the Study of Human Activities"....July 18.
W. J. V. OSTERHOUT..."Some Quantitative Researches on the Permeability of Plant Cells".July 22.
E. S. MORSE....."Mars and its Mysteries".....July 25.
W. E. GARREY....."Some Aspects of the Physiology of Cardiac Nerves".....July 29.
C. H. EIGENMANN...."The Panama Problem and a Trip Through Colombia, South America".....August 1.
C. E. MCCLUNG....."Chromosome Individuality".....August 5.
C. R. STOCKARD....."Injurious Treatments of the Male, and the Influence on the Offspring".....August 8.
CHARLES T. BRUES and M. J. ROSENAU....."The Relation of the Stable Fly (*Stomaxys calcitrans*) to the Transmission of Infantile Paralysis"..August 12.

6. MEMBERS OF THE CORPORATION

1. LIFE MEMBERS, 1912.

ALLIS, MR. E. P., JR., Palais Carnoles, Menton, France.
ANDREWS, MRS. GWENDOLEN FOULKE, Baltimore, Md.
BILLINGS, MR. R. C., 66 Franklin St., Boston, Mass.
CAREY, MR. ARTHUR ASTOR, Fayerweather St., Boston, Mass.
CLARKE, PROF. S. F., Williams College, Williamstown, Mass.
CONKLIN, PROF. EDWIN G., Princeton University, Princeton, N. J.

CRANE, MR. C. R., 2559 Michigan Blvd., Chicago, Ill.
DAVIS, MAJOR HENRY M., Syracuse, N. Y.
EVANS, MRS. GLENDOWER, 12 Otis Place, Boston, Mass.
FARLOW, PROF. W. G., Harvard University, Cambridge, Mass.
FAY, MISS S. B., 88 Mt. Vernon St., Boston, Mass.
FOLSOM, MISS AMY, 88 Marlboro St., Boston, Mass.
FOOT, MISS KATHERINE, 80 Madison Ave., New York City, N. Y.
GARDINER, MRS. E. G., Woods Hole, Mass.
GARDINER, MISS EUGENIA, 15 W. Cedar St., Boston, Mass.
HANNAMAN, MR. CHARLES E., 103 1st St. Troy, N. Y.
HARRISON, EX-PROVOST C. C., University of Pennsylvania, Philadelphia, Pa.
JACKSON, MISS M. C., 88 Marlboro St., Boston, Mass.
JACKSON, MR. CHAS. C., 24 Congress St., Boston, Mass.
KENNEDY, MR. GEO. G., 284 Warren St., Roxbury, Mass.
KIDDER, MR. C. G., 27 William St., New York City, N. Y.
KIDDER, MR. NATHANIEL T., Milton, Mass.
KING, MR. CHAS. A.,
LEE, MRS. FREDERIC S., 279 Madison Ave., New York City, N. Y.
LOWELL, MR. A. LAWRENCE, 171 Marlboro St., Boston, Mass.
MARRS, MRS. LAURA NORCROSS, 9 Commonwealth Ave., Boston, Mass.
MASON, E. F., 1 Walnut St., Boston, Mass.
MASON, MISS IDA M., 1 Walnut St., Boston, Mass.
MEANS, MR. JAMES HOWARD, 196 Beacon St., Boston, Mass.
MERRIMAN, MRS. DANIEL, Worcester, Mass.
MINNS, MISS SUSAN, 14 Louisburg Square, Boston, Mass.
MINNS, MR. THOMAS, 14 Louisburg Square, Boston, Mass.
MINOT, DR. CHAS. S., Harvard Medical School, Boston, Mass.
MIXTER, MISS M. C., 241 Marlboro St., Boston, Mass.
MORGAN, MR. J. PIERPONT, JR., Wall and Broad Sts., New York City, N. Y.
MORGAN, PROF. T. H., Columbia University, New York City, N. Y.
MORGAN, MRS. T. H., New York City, N. Y.
NOYES, MISS EVA J., 28 South Willow St., Montclair, N. J.
NUNN, MR. LUCIAN L., Telluride, Colo.
OSBORN, PROF. HENRY F., American Museum of Natural History, New York.

PHILLIPS, DR. JOHN C., 299 Berkeley St., Boston, Mass.
PHILLIPS, MRS. JOHN C., 299 Berkeley St., Boston, Mass.
PORTER, DR. H. C., University of Pennsylvania, Philadelphia, Pa.
PULSIFER, MR. W. H., Newton Center, Mass.
ROGERS, MISS A. P., 5 Joy St., Boston, Mass.
SEARS, DR. HENRY F., 420 Beacon St., Boston, Mass.
SHEDD, MR. E. A.,
SMITH, MRS. C. C., 286 Marlboro St., Boston, Mass.
STROBELL, MISS E. C., 80 Madison Ave., New York City, N. Y.
THORNDIKE, DR. EDWARD L., Teacher's College, Columbia University, New York City, N. Y.
TRELEASE, PROF. WILLIAM, University of Illinois, Champaign, Ill.
WARE, MISS MARY L., 41 Brimmer St., Boston, Mass.
WARREN, MRS. S. D., 67 Mt. Vernon St., Boston, Mass.
WHITNEY, MR. HENRY M., Brookline, Mass.
WILLCOX, MISS MARY A., Wellesley College, Wellesley, Mass.
WILMARTH, MRS. H. D., Elliott St., Jamaica Plain, Mass.
WILLIAMS, MRS. ANNA P., 505 Beacon St., Boston, Mass.
WILSON, DR. E. B., Columbia University, New York City, N. Y.
WILSON, PROF. W. P., Philadelphia Museum, Philadelphia, Pa.

2. MEMBERS, AUGUST, 1913

ABBOTT, PROF. J. F., Washington University, St. Louis, Mo.
ABBOTT, MISS MARGARET B., 413 W. Front Street, Plainfield, N. J.
ADAMS, Dr. C. F., University of Arkansas, Fayetteville, Ark.
ADDISON, DR. W. H. F., Univ. of Pennsylvania, Med. Sch., Philadelphia.
ADKINS, MR. W. S., Department of Zoölogy, Columbia University, New York City.
ALLEN, PROF. EZRA, School of Pedagogy, Philadelphia, Pa.
ALLYN, DR. HARRIET M.,
ALSBURG, DR. C. S., U. S. Department of Agriculture, Washington, D. C.

BAITSELL, DR. GEORGE A., Sheffield Scientific School, Yale University, New Haven, Conn.

BAKER, DR. E. H., 5727 Kimbark Avenue, Chicago, Illinois.
BANCROFT, DR. F. W., Rockefeller Institute, New York City.
BARDEEN, PROF. C. R., Univ. of Wisconsin, Madison, Wis.
BECKWITH, MISS CORA J., Vassar College, Poughkeepsie, N. Y.
BEHRE, MISS ELINOR H., Sophie Newcomb College, Tulane Univ., New Orleans.
BEYER, DR. H. C., Stoneleigh Court, Washington, D. C.
BIGELOW, PROF. M. A., Teachers' College, Columbia University, N. Y. C.
BIGELOW, PROF. R. P., Mass. Inst. Technology, Boston, Mass.
BINFORD, DR. RAYMOND, Guilford College, S. C.
BOX, MISS CORA MAY, Univ. of Cincinnati, Cincinnati, O.
BRADLEY, DR. HAROLD S., Univ. of Wisconsin, Madison, Wis.
BROWNE, MISS ETHEL N., Dana Hall, Wellesley College, Wellesley, Mass.
BUCKINGHAM, MISS EDITH N., 342 Marlboro Street, Boston, Mass.
BUDINGTON, PROF. R. A., Oberlin College, Oberlin, Ohio.
BUMPUS, DR. H. C., University of Wisconsin, Madison, Wis.
BYRNES, DR. ESTHER F., 193 Jefferson Avenue, Brooklyn, N. Y.

CALKINS, PROF. GARY N., Columbia Univ., New York City.
CALVERT, PROF. PHILIP P., Univ. of Pennsylvania, Philadelphia.
CARLSON, PROF. A. J., Univ. of Chicago, Chicago, Ill.
CARVER, MR. GAIL L., Mercer Univ., Macon, Ga.
CARY, DR. L. R., Princeton Univ., Princeton, N. J.
CATTELL, PROF. J. MCKEEN, Garrison-on-Hudson, New York.
CHAMBERS, DR. ROBERT, Univ. of Cincinnati, Cincinnati, Ohio.
CHEESEMAN, DR. T. M., Garrison, N. Y.
CHESTER, PROF. WEBSTER, Colby College, Waterville, Me.
CHIDESTER, DR. F. E., Rutgers College, New Brunswick, N. J.
CHILD, PROF. C. M., Univ. of Chicago, Chicago, Ill.
CLAPP, PROF. CORNELIA M., Mt. Holyoke College, South Hadley, Mass.
CLARK, DR. E. R., Johns Hopkins Univ., Baltimore, Md.
COE, PROF. W. R., Yale University, New Haven, Conn.
COLTON, PROF. H. S., Ardmore, Pa.
COOK, MISS DELLAR L., 28 Andrews St., Woonsocket, R. I.
COMSTOCK, PROF. J. H., Cornell University, Ithaca, N. Y.

COOLIDGE, MR. C. A., Ames Building, Boston, Mass.
CRAMPTON, PROF. H. E., Barnard College, Columbia Univ., New York City.
CRANE, MRS. C. R., 1559 Michigan Boulevard, Chicago, Ill.
CURTIS, PROF. W. C., University of Missouri, Columbia, Mo.

DERICK, PROF. CARRIE M., McGill Univ., Montreal, Canada.
DEXTER, MR. H. S., Northland College, Ashland, Wis.
DODDS, PROF. G. S., Univ. of Missouri, Columbia, Mo.
DONALDSON, DR. H. H., Wistar Institute of Anat. and Biol., Philadelphia.
DORRANCE, MISS ANN, Dorranceton, Pa.
DORRANCE, MISS FRANCES, Dorranceton, Pa.
DREW, PROF. GILMAN A., Marine Biological Laboratory, Woods Hole, Mass.
DUGGAR, PROF. B. M., Cornell Univ., Ithaca, New York.
DUNGAY, DR. NEIL S., Carleton College, Northfield, Minn.

EATON, PROF. E. H., Hobart College, Geneva, New York.
EDWARDS, MR. D. J., College of the City of New York, New York City.
EIGENMANN, PROF. C. H., Univ. of Indiana, Bloomington, Ind.
EWALD, DR. W. F., Rockefeller Institute, New York City.

FARNAM, MISS LOUISE W., 43 Hillhouse Avenue, New Haven, Conn.
FERGUSON, PROF. J. S., Cornell Univ. Medical School, New York City.
FIELD, MISS HAZEL E., Belhaven College, Jackson, Miss.
FIELD, PROF. IRVING, 5 Enfield Street, Worcester, Mass.
FISH, MR. J. BURTON, 883 Freeman Street, New York City.
FLANIGEN, MISS RUTH, Woodbury, New Jersey.
FURST, MR. CLYDE, Carnegie Foundation, 576 Fifth Ave., New York City.

GAGE, PROF. S. H., Cornell Univ., Ithaca, New York.
GARREY, PROF. W. E., Washington Univ., St. Louis, Mo.
GIES, PROF. W. J., Columbia Univ., Dept. Physiological Chemistry, New York.
GLASER, PROF. O. C., Univ. of Michigan, Ann Arbor, Mich.

GLASER, MR. R. W., Bussey Institution, Forest Hills, Boston, Mass.

GOLDFARB, PROF. A. J., College of the City of New York, New York City.

GOODRICH, MR. H. B., Columbia Univ., Dept. Zoology, New York City.

GRAVE, DR. CASWELL, Johns Hopkins Univ., Baltimore, Md.

GREGORY, DR. LOUISE H., Barnard College, Columbia Univ., New York City.

GREENMAN, Dr. M. J., Wistar Institute of Anat. and Biol., Philadelphia.

GUNTHER, MISS MAUDE C., Eastern High School, Washington, D. C.

HARPER, PROF. R. A., Columbia Univ., New York City.

HAHN, Dr. C. W., High School of Commerce, New York City.

HALL, PROF. ROBERT W., Lehigh University, South Bethlehem, Pa.

HANCE, MR. ROBERT T., Univ. of Cincinnati, Cincinnati, Ohio.

HARGITT, DR. C. W., Syracuse Univ., Syracuse, New York.

HARRISON, MR. A. C., 660 Drexel Building, 5th & Chestnut Sts., Philadelphia.

HARMAN, DR. MARY T., Kansas State Agricultural College, Manhattan, Kans.

HARRISON, PROF. ROSS G., Yale University, New Haven, Conn.

HARVEY, PROF. B. C. H., Univ. of Chicago, Chicago, Ill.

HARVEY, DR. E. N., Princeton Univ., Princeton, N. J.

HAYDEN, MISS MARGARET A., Carnegie Institute of Technology, Pittsburgh.

HAYES, PROF. S. P., Mount Holyoke College, South Hadley, Mass.

HEATH, PROF. HAROLD, Stanford University, Calif.

HEGNER, PROF. R. W., Univ. of Michigan, Ann Arbor, Mich.

HOAR, MR. D. BLAKELY, 161 Devonshire Street, Boston, Mass.

HOGE, MISS MILDRED A., 509 W. 121st Street, New York City.

HOGUE, DR. MARY J., Mt. Holyoke College, South Hadley, Mass.

HOLMES, PROF. S. J., Univ. of California, Berkeley, Calif.

ISAACS, MR. RAPHAEL, Univ. of Cincinnati, Cincinnati, Ohio.

ISELEY, PROF. F. B., Central College, Fayette, Mo.

JACKSON, PROF. C. M., Univ. of Missouri, Columbia, Mo.
JACOBS, MR. MURKEL H., Univ. of Pennsylvania, Zool. Lab., Philadelphia.
JENNINGS, PROF. H. S., Johns Hopkins University, Baltimore, Md.
JENNER, PROF. E. A., Simpson College, Indianola, Iowa.
JONES, PROF. LYNDS, Oberlin College, Oberlin, Ohio.
JUST, PROF. E. E., Howard University, Washington, D. C.

KELLEY, MR. F. J., University of Wisconsin, Madison, Wis.
KELLICOTT, PROF. W. E., Goucher College, Baltimore, Md.
KELLY, MR. J. P., 2163 Gleason Avenue, Unionport, New York.
KENNEDY, DR. HARRIS, Readville, Mass.
KING, DR. HELEN DEAN, Wistar Institute, Philadelphia, Pa.
KINGSBURY, PROF. B. F., Cornell Univ., Ithaca, New York.
KINGSLEY, PROF. J. S., Univ. of Illinois, Urbana, Ill.
KIRKHAM, DR. W. B., Yale University, New Haven, Conn.
KNIGHT, MISS MARIAN V., Smith College, Northampton, Mass.
KNOWER, PROF. H. McE., Univ. of Cincinnati, Cincinnati, Ohio.
KNOWLTON, PROF. F. P., Syracuse University, Syracuse, New York.
KNUDSON, PROF. LEWIS, Cornell University, Ithaca, N. Y.
KRIBS, DR. HERBERT, Univ. of Pennsylvania, Philadelphia, Pa.

LAMBERT, DR. R. A., Columbia Univ., Dept. Pathology, New York City.
LEE, PROF. F. S., 437 West 59th Street, New York City.
LEFEVRE, PROF. GEORGE, Univ. of Missouri, Columbia, Mo.
LEWIS, PROF. I. F., Univ. of Wisconsin, Madison, Wis.
LEWIS, MRS. W. H., 1931 East 31st St., Baltimore, Md.
LEWIS, PROF. W. H., Johns Hopkins University, Baltimore, Md.
LILLIE, PROF. FRANK R., University of Chicago, Chicago, Ill.
LILLIE, PROF. R. S., Clark University, Worcester, Mass.
LINTON, PROF. EDWIN, Washington and Jefferson College, Washington, Pa.
LOEB, PROF. JACQUES, Rockefeller Institute, New York City.
LOEB, DR. LEO, St. Louis Skin and Cancer Hospital, St. Louis, Mo.
LOWTHER, MRS. FLORENCE DEL., Barnard College, Columbia Univ., New York.

LUSCOMBE, MR. W. O., Woods Hole, Mass.
LYMAN, PROF. GEORGE R., Dartmouth College, Hanover, N. H.
LYNCH, MISS CLARA J., Smith College, Northampton, Mass.
LYON, DR. E. P., St. Louis Univ. Medical School, St. Louis, Mo.
LUND, MR. E. J., Johns Hopkins University, Baltimore, Md.

MCCLENDON, DR. J. F., Cornell University Medical School, New York City.
MCCLUNG, PROF. C. E., Univ. of Pennsylvania, Philadelphia, Pa.
MCGILL, DR. CAROLINE, Murray Hospital, Butte, Montana.
MCGREGOR, DR. J. H., Columbia University, New York City.
MCINDOO, DR. N. E., Bureau of Entomology, Washington, D. C.
MACKENZIE, PROF. MARY D., Carnegie Institute Technology, Pittsburgh, Pa.
MCMURRICH, PROF. J. P., University of Toronto, Toronto, Canada.
MALL, PROF. F. P., Johns Hopkins University, Baltimore, Md.
MALONE, DR. E. F., Univ. of Cincinnati, Cincinnati, Ohio.
MARTIN, MISS BERTHA E., University of Chicago, Dept. Zool., Chicago, Ill.
MARQUETTE, MR. WILLIAM G., Columbia Univ., New York City.
MAST, PROF. S. O., Johns Hopkins Univ., Baltimore, Md.
MATHEWS, PROF. A. P., Univ. of Chicago, Chicago, Ill.
MAYER, DR. A. G., Maplewood, N. J.
MEIGS, DR. E. B., Radnor, Pa.
MELTZER, DR. S. J., 107 West 122d Street, New York City.
METCALF, PROF. M. M., Oberlin College, Oberlin, Ohio.
MINOR, MISS MARIE L., Wadleigh High School, New York City.
MORGAN, PROF. H. A., Agricultural Experiment Station, Knoxville, Tenn.
MOORE, Prof. G. T., Missouri Botanical Gardens, St. Louis, Mo.
MOORE, PROF. J. PERCY, Univ. of Pennsylvania, Philadelphia, Pa.
MORRILL, PROF. A. D., Hamilton College, Clinton, New York.
MORRILL, DR. C. V., New York University, New York City.
MURBACH, Dr. L., Poultney, Vermont.

NACHTRIEB, PROF. HENRY F., Univ. of Minnesota, Minneapolis, Minn.

NEAL, PROF. H. V., Tufts College, Mass.
NEWMAN, PROF. H. H., University of Chicago, Chicago, Ill.
NICHOLS, DR. M. LOUISE, 3207 Summer Street, Philadelphia, Pa.
NOWLIN, MISS NADINE, Univ. of Kansas, Lawrence, Kans.

OLMSTED, PROF. J. M. D., Richmond College, Richmond, Va.
OSBURN, PROF. R. C., 557 West 124th Street, New York City.
OSTERHOUT, PROF. W. J. V., Harvard University, Cambridge, Mass.

PACKARD, DR. CHARLES, Columbia Univ., Dept. Zool., New York City.
PACKARD, DR. W. H., Bradley Polytechnic Institute, Peoria, Ill.
PAINTER, MR. T. S., Yale University, New Haven, Conn.
PAPPENHEIMER, DR. A. M., Columbia Univ., Dept. Pathology, New York City.
PARKER, PROF. G. H., 16 Berkeley Street, Cambridge, Mass.
PATON, DR. STEWART, Princeton Univ., Princeton, N. J.
PATTEN, MISS J. B., Elm Brook, South Natick, Mass.
PATTEN, DR. WILLIAM, Dartmouth College, Hanover, N. H.
PATTERSON, PROF. J. T., University of Texas, Austin, Texas.
PAYNE, PROF. FERNANDUS, Univ. of Indiana, Bloomington, Ind.
PEARSE, PROF. A. S., Univ. of Wisconsin, Madison, Wis.
PIKE, PROF. FRANK H., 437 West 59th St., New York City.
PRENTISS, MISS HENRIETTA, Normal College, New York City.

QUACKENBUSH, MR. L. S., 27 West 73d Street, New York City.

RANKIN, PROF. W. M., Princeton Univ., Princeton, N. J.
REA, DR. PAUL M., Charleston Museum, Charleston, S. C.
REIGHARD, PROF. JACOB, Univ. of Michigan, Ann Arbor, Mich.
REINKE, MR. E. E., Princeton Univ., Princeton, N. J.
RICE, PROF. EDWARD L., Wesleyan Univ., Delaware, Ohio.
ROBBINS, MR. W. J., Cornell Univ., Ithaca, New York.
ROBERTS, MISS EDITH A., Mount Holyoke College, South Hadley, Mass.
ROBERTSON, PROF. W. R. B., Univ. of Kansas, Lawrence, Kans.
ROGERS, PROF. CHARLES G., Syracuse Univ., Syracuse, N. Y.
ROSENOW, DR. E. C., People's Gas Building, Chicago, Ill.
RUDDIMAN, MISS MARGUERITE, 441 Senator Street, Brooklyn, New York.

SANDS, MISS ADELAIDE G., 348 N. Main St., Port Chester, New York.

SANDS, DR. GEORGIANA, 348 N. Main Street, Port Chester, New York.

SCOTT, PROF. G. G., College of the City of New York, New York City.

SCOTT, PROF. JOHN W., University of Wyoming, Laramie, Wyo.

SHOREY, DR. MARIAN L., Milwaukee-Downer College, Milwaukee, Wis.

SHULL, DR. A. FRANKLIN, University of Michigan, Ann Arbor, MICH.

SMITH, DR. BERTRAM G., State Normal College, Ypsilanti, Mich.

SOLLMAN, DR. TORALD, Western Reserve University, Cleveland, Ohio.

SPAULDING, PROF. E. G., Princeton Univ., Princeton, N. J.

SPENCER, DR. H. J., 1124 Amsterdam Avenue, New York City.

STEWART, MISS MARY W., Barnard College, Columbia Univ., New York City.

STOCKARD, PROF. C. R., Cornell Medical College, New York City.

STREETER, DR. GEORGE L., University of Michigan, Ann Arbor, Mich.

STRONG, DR. O. S., 437 West 59th St., New York City.

STRONG, DR. R. M., University of Chicago, Chicago, Ill.

STURTEVANT, MR. A. H., Columbia Univ., Dept. Zool., New York City.

SUMNER, DR. F. B., Sausalito, Calif., Albatross.

TASHIRO, DR. SHIRO, University of Chicago, Chicago, Ill.

TAYLOR, MISS KATHARINE A., 1304 Eutaw Place, Baltimore, Md.

TENNENT, PROF. D. H., Bryn Mawr College, Pa.

TERRY, PROF. O. P., Indiana Medical School, W. Lafayette, Ind.

THOMAS, DR. ADRIAN, 2517 Hanover Avenue, Richmond, Va.

THOMPSON, PROF. CAROLINE B., 195 Weston Road, Wellesley, Mass.

TINKHAM, MISS FLORENCE L., 56 Temple Street, Springfield, Mass.

TOMPKINS, MISS ELIZABETH M., 2019 Bedford Avenue, Brooklyn, N. Y.

TREADWELL, PROF. A. L., Vassar College, Poughkeepsie, N. Y.
TURNER, MR. C. L., Ohio Wesleyan Univ., Delaware, Ohio.

USHER, MISS SASANNAH, 1007 West Illinois St., Urbana, Ill.

VAN CLEAVE, DR. H. J., University of Illinois, Urbana, Ill.
VAUGHAN, DR. T. C., U. S. Geological Survey, Washington, D. C.

WAITE, PROF. F. C., Western Reserve Univ. Medical School, Cleveland, O.
WALKER, DR. GEORGE, Charles & Center Streets, Baltimore, Md.
WALLACE, PROF. LOUISE B., Mt. Holyoke College, South Hadley, Mass.
WARD, PROF. H. B., Univ. of Illinois, Urbana, Ill.
WARREN, PROF. HOWARD C., Princeton Univ., Princeton, N. J.
WASTENEYS, MR. HARDOLPH, Rockefeller Institute, New York City.
WATSON, MR. FRANK E., 218 Washington Street, Geneva, New York.
WATSON, PROF. J. B., Johns Hopkins University, Baltimore, Md.
Wheeler, PROF. W. M., Bussey Institution, Forest Hills, Boston, Mass.
WHEELER, MISS ISABEL, The Hattersley, Toledo, Ohio.
WHERRY, DR. W. B., Cincinnati Hospital, Cincinnati, Ohio.
WHITNEY, DR. DAVID D., Wesleyan Univ., Middletown, Conn.
WERBER, DR. E. I., 1504 E. 62d Street, Chicago, Ill.
WIEMAN, PROF. H. L., Univ. of Cincinnati, Cincinnati, Ohio.
WILCOX, DR. ALICE W., 56 Alumni Avenue, Providence, R. I.
WILDMAN, DR. E. E., 4331 Osage Avenue, Philadelphia, Pa.
WILLIAMS, DR. ANNA W., 549 Riverside Drive, New York City.
WILSON, PROF. H. V., University of North Carolina, Chapel Hill, N. C.
WOGLOM, DR. WILLIAM H., Columbia University, New York City.
WOLFE, PROF. JAMES J., Trinity College, Durham, N. C.
WOODRUFF, PROF. L. L., Yale University, New Haven, Conn.
WRIGHT, PROF. R. RAMSAY, University of Toronto, Toronto, Canada.

YERKES, PROF. R. M., Harvard University, Cambridge, Mass.
YOUNG, MR. D. B., Amherst College, Amherst, Mass.

THE MIGRATION OF THE GERM CELLS IN AMIURUS NEBULOSUS.

FREDA M. BACHMANN.

A study of the germ cells in *Amiurus nebulosus* was undertaken with the hope of determining their origin, but because of the difficulties in distinguishing them in the younger stages of the embryo and the limited amount of time in which this work was done, it was necessary to limit the subject to a study of their number and migration after they are clearly distinguishable.

The material was fixed in either Zenker's fluid or bichromate acetic. The two fluids appear to give equally good results. All sections were cut 10 microns thick and stained with Heidenhain's iron-alum hematoxylin followed by Congo red or with Mayer's hæmalum followed by Orange G.

The origin and migration of the sex cells and the formation of the germ gland in teleosts have been studied in a number of species by several investigators. Some have concluded that the germ cells are all or in part transformed epithelial cells; others believe they are never a part of the body tissues but are derived from undifferentiated cells in the very early stages of development and later migrate into the sex gland where by division, they give rise to oogonia and spermatogonia.

Nussbaum ('80) in working on the trout, found no evidence of any epithelial cells transformed into germ cells. At a certain stage in development, the germ cells divide and form little groups or nests of cells. He thought these could not be transformed epithelial cells because if the cells in the groups are of epithelial origin, then why should they be in groups, and also, if epithelial cells are continually being transformed into germ cells, then why, when these smaller cells appear in groups, do the larger cells which were in stages immediately preceding so suddenly and completely disappear? Also he found if there was a large number of cells in a nest the individual cells were smaller than those in nests where there were but a few cells. He thought the size of

the individual cells in a nest in proportion to the size of the nest, good proof that no epithelial cells are transformed into germ cells. The nests of cells are at first some distance apart and become nearer together as the cells increase in number by division —not as new cells are formed from epithelial cells.

MacLeod ('81) in *Hippocampus* and *Belone acus* observed that the sex cells appear in the somatopleure and splanchopleure rather late in development and thought them to be differentiated peritoneal cells. He thought the genital fold originates in a group of germ cells on the surface of the epithelium.

Hoffmann ('86) studied salmon embryos. According to him the genital glands have their origin in the primary germ cells which are first found in the median and dorsal part of the mesentery and laterally to the body wall. He could see nothing more in these germ cells than transformed and highly differentiated peritoneal cells which in the youngest stages of development increase in number by mitosis. Later these cells come to lie laterally from the mesentery and extend into the body cavity as a small fold. The cells increase in number by division and transformation of more peritoneal cells. Nests of germ cells are thus formed. Later each cell becomes surrounded by follicle cells. He could not say whether these follicle cells are connective tissue cells or of epithelial origin, *i. e.*, from a germinal epithelium. However, he inclined to the latter because the follicle cells appear before a connective tissue stroma is differentiated.

Böhi ('04) worked on the trout and the salmon. In the trout, twenty-five days after fertilization and six days later in the salmon, he found large cells among the larger epithelial cells in both the somatopleure and splanchopleure. The cells have very large nuclei but no nucleoles. The cytoplasm stains lightly and is without definite structure. The nuclear content is granular and hence takes little of the stain. The cells are more oval or ovoid in younger embryos and rounder in older ones. There is a diminution in volume before division begins. There is a definite relation between the size of the nucleus and the size of the cell—the large cells always have the larger nuclei. In two trout embryos of twenty-five days he found in the lateral plate four and six germ cells respectively. In salmon embryos of

thirty-one days he found germ cells in the same position. After the cœlome is formed he found the cells in both somatopleure and splanchnopleure—in one embryo 13 in the somatopleure, 4 in the splanchnopleure and 3 between these layers.

In salmon embryos the number of germ cells remains constant up to 185 days after fertilization. But this number varies from 20 to 54. In an embryo of 154 days he found 46 germ cells, in one of 185 days, 234 cells, in one of 199 days, 373 cells. The most posterior germ cell is six or seven somites anterior to the anus. As the embryo develops the nuclei enlarge to some extent so that the ratio of the diameter of the nucleus to that of the cell becomes somewhat greater. The nucleus has a sharper contour, loses its granular appearance and shows small chromatin bodies. From 46 to 50 days after fertilization the first small nucleoles appear, later they are larger, stain more deeply and increase in number.

The genital ridge arises as a discontinuous thickening of the cœlomic epithelium in the region of a germ cell. The thickenings unite and others are formed anteriorly and posteriorly. The uniting of these thickenings takes place at about 60 days. By 82 days the ridge is continuous in the gonal part of the embryo and is represented in the progonal and epigonal region by separate thickenings. The end of the genital ridge is posterior to the anus in one salmon embryo. The genital fold rises discontinuously. The cœlome cells of the genital ridge pull apart and leave a space in which there is a fluid. This fold extends progonally and epigonally and ends in the genital ridge.

In agreement with Nussbaum and Jungersen, Böhi found the genital fold to be composed of epithelial cells and not as MacLeod had observed it—a group of germ cells on the surface of the epithelium. Böhi believed the cœlome cells to give rise to (*a*) indifferent cells, (*b*) follicle cells, (*c*) germ cells. The cells on the sides of the genital fold gradually become transformed into germ cells. This changing of epithelial cells into germ cells lasts for only a short time and in embryos of 277 days no more transition cells can be found. The epithelial cells next to the germ cells are converted into follicle cells by pressure of the germ cells against them. According to Böhi some of the germ cells are

differentiated early in development while others arise from epithelial cells after the genital fold is formed.

Eigenmann ('92) in studying *Micrometrus aggregatus* found that the sex cells first become conspicuous in the mesoblast at a stage before any protovertebræ are formed. They can be seen in earlier stages but do not stand out so prominently from the other cells. Judging from their size they are probably segmentation cells of the fifth generation. The sex cells can first be distinguished about the time the blastopore closes. The earliest ones are before the mesoderm is split off from the entoderm. They differ from the surrounding cells in having well-defined rounded outlines and in the distribution of the chromatin in the nucleus. In the sex cells the chromatin is uniformly distributed in small granules; in the surrounding cells it is collected in two or three masses. Not all the eggs of this stage show the sex cells equally well. In some eggs in which the blastopore is closing none can be made out with certainty. In some eggs many large cells were seen in the ectoderm but the inner cells of the blastoderm segment more rapidly toward the close of segmentation than the outer cells and these outer ones become distended through intracellular digestion of the surrounding ovarian fluid. But it is still possible that the sex cells arise in the ectoderm. If they are segregated as early as the fifth generation, *i. e.*, before there is any differentiation into ectoderm and entoderm, it seems to be of no great importance whether at the time of the separation of the blastoderm into ectoderm and entoderm the cells lie in its inner or outer portion. There is no change in number or size up to the time the larvæ are 2.5 mm. long. The length over which the majority of cells is distributed is about 0.20 mm.

Eigenmann ('96) continued his observations on the sex cells in *Micrometrus aggregatus*. He finds that the striking feature is the asymmetry of the two sides and the variation in number and position of the cells in different larvæ. He finds the nuclei in the sex cells of embryos 5 mm. in length have become somewhat larger and the nucleoles also so that in the period of apparent inactivity there have been histogenic changes just as truly as the changes in the somatic cells although there is no division. In later stages the tissues containing the sex cells form a median

ridge in the dorsal end of the mesentery. This ridge divides anteriorly into two ridges each of which end in a single little fold of the peritoneum which is differentiated only by being rich in nuclei. In some larvæ at this time the germ cells have not increased in number, in others they may have divided once. In one larva in which a few cells were widely separated from the majority, the ridge entirely disappeared to reappear in the neighborhood of some widely separated anterior cells. From such instances it is evident that the germinal ridge develops only under the influence of the reproductive cells.

Dodds ('10) in *Lophius* was able to recognize germ cells when the blastoderm had not quite half covered the yolk. At this time they are in the primary entoblast. They pass into the mesoblast when this is separated and into that part which becomes the myotome. Later when the mesoblast is separated into myotome and lateral plate the germ cells migrate into the latter and again when this splits they are left in the splanchnopleure. Later they migrate toward the median border of the cœlome and thence upward into the somatic layer and to the position of the permanent germ cells.

The number of germ cells varies but is small—not over fifty-five. From the time they are recognizable until they are in the position of the germ gland there is no increase in number. The apparent increase in early stages is due to changes in the cells which make them more easily recognizable. The cells are characterized by rounded outlines or they may be amœboid. The cytoplasm stains more deeply than that of the somatic cells, the nucleus is smaller, irregular in shape, apparently less turgid and contains two small nucleoles. The nuclei of the somatic cells have two large nucleoles or a single very large nucleole. The decrease in the size of the germ cell nucleoles is due to a loss of part of their content through the nuclear membrane into the cytoplasm. This extrusion of nuclear material does not take place simultaneously from the nucleoles of any one nucleus nor from all germ cells.

There is no segmental arrangement of the germ cells. There is both an active migration of the cells and a passive change due to growth of the surrounding tissue. Dodds is of the opinion

that before any differences between germ cells and somatic cells can be detected there must be an unseen physiological difference which determines the future behavior of the cells.

The eggs of *Amiurus nebulosus* vary in size in the different nests. There is often a difference of 1 mm. in diameter. For this reason it follows that embryos of the same length are not necessarily of the same stage of development. Embryos 3.2 mm. long taken from one nest may be slightly further developed than those 3.7 mm. long taken from another nest.

When stained with iron hematoxylin the yolk granules of the egg are black but if this is washed out with iron alum and then followed by congo red the granules are a deep red. The yolk material in the germ cells is more or less diffused through the cytoplasm and loses the black hematoxylin stain quite readily in the iron alum. But because of the contained yolk the cytoplasm of the germ cells stains a deeper red than the cytoplasm of the surrounding cells. Occasionally there is a small compact mass of yolk in a germ cell which retains the black stain. Yolk granules of the egg stain lightly with Mayer's hæmalum but take up the Orange G very rapidly. Hence the cytoplasm of the germ cells stains a light orange with hæmalum and Orange G. The cytoplasm of the surrounding cells is a bluish gray.

The germ cells in all of the stages studied up to the time of multiplication were found to be about the same size. They are from 14 to 18 microns in diameter and the nuclei 7 to 9 microns in diameter. The germ cells are quite well rounded though sometimes they are more or less amœboid. The cytoplasm is quite clear, especially so if the contained yolk is in one or two compact masses. The nucleus is spherical or frequently lobed. But this lobed condition of the nucleus is not peculiar to the nuclei of germ cells—it is often found in nuclei of other cells. There are often two nucleoles but again this is no peculiarity of germ cell nuclei. In the very early stages of development most of the somatic cells have more than one nucleole and two nucleoles are seen in the nuclei of many somatic cells long after the cœlomic cavity is formed. Not infrequently there are no nucleoles in the germ cell nuclei. The chromatin which is fairly distinct is scattered through the nucleus in the form of fine granules. It

is never aggregated into large masses as is common in the nuclei of somatic cells.

The germ cells with few exceptions have been found in the mesoderm. These exceptions were a few cells in the cœlomic cavity or between the lateral plate of mesoderm and the yolk, also a few doubtful cells in the entoderm. In younger embryos the germ cells are in the lateral plate of the mesoderm and near the region where the blastopore has closed. In older embryos where the tail has grown out beyond the yolk they are in the splanchnopleure surrounding the hind gut. Later they are in the mesentery and in the germ gland anlage. The exact location of the germ cells will be given in greater detail in the description of the different stages studied.

The number of germ cells is constant until after or about the time they are in the germ gland anlage. The average number is about 23 with extremes 12 and 34. Because of the large size of the germ cells and the thickness of the sections it would be possible to count the same cells twice. However, by noting the position and appearance of the cells and the size of the nucleus, it is believed that few errors have been made in counting.

There is probably no one characteristic peculiar to germ cells in *Amiurus nebulosus*. Blood corpuscles are frequently nearly spherical. But in these the cytoplasm stains more lightly with congo red and Orange G., the nucleoles are larger, and the nuclear membrane much more distinct than in the germ cells. Also the ratio of the diameter of the nucleus to the diameter of the cell is greater in blood corpuscles than the same ratio in germ cells. In a number of blood corpuscles this ratio was 2 : 3, in germ cells it is 1 : 2. The cytoplasm of the entoderm cells often contains much yolk and hence these frequently stain like germ cells. But the entoderm cells are usually smaller and columnar or irregular in shape, and the nuclei are more often oval than spherical with two or three large nucleoles. In the earliest stage studied the mesoderm cells were not more than half the size of the germ cells and contained little if any yolk. Even in this stage the nuclei of the mesoderm cells are becoming oval or elongated. In later stages the mesoderm cells are small, very irregular in shape with cytoplasmic processes. The germ cells

are easily recognized in any stage by their shape, size, and staining qualities.

Embryos 3.2–3.7 mm. Long.

In embryos of this length the blastopore is closed and the tail extends about 1 mm. beyond the yolk. The pronephric ducts have been formed. There is no trace of a split in the mesoderm which is to give rise to the cœlomic cavity. The axial mesoderm is a compact mass of cells extending laterally and ventrally and composed of cells which are still quite regular in outline although the nuclei are more or less oval. There is scarcely a trace of yolk in these mesodermal cells. The entoderm cells frequently contain large yolk masses or have yolk material diffused throughout the cytoplasm.

The germ cells are found in the lateral plate of mesoderm in the region just anterior to where the tail leaves the yolk. The germ cells may be anywhere in the lateral plate but the majority of them were found some distance away from the mid-line (Fig. 1). Not infrequently they are far out in the lateral plate as shown in Fig. 2. A few germ cells were found between the yolk and the mesoderm. It may be possible that they came from the yolk but at least none were seen in the yolk nor coming out of it. The germ cells are so very different in appearance from the other somatic cells that there is no danger in overlooking them in well-stained sections. The length of the region in which they are found varies from 0.025 mm. to 0.054 mm. The embryos are not symmetrical with regard to the number of germ cells found on each side. The total number of an embryo is from 12 to 28. The following table shows the number found on each side, the total number, and the distance throughout which the germ cells were distributed in several of the embryos sectioned.

	Right.	Left.	Total.	Length of Region, Mm.
1.....	15	8	23	0.250
2.....	8	14	22	0.260
3.....	10	9	19	0.360
4.....	14	13	28	0.250
5.....	4	9	13	0.340
6.....	12	13	26	0.640

In number 4, one cell was in the entoderm, and in number 6, a germ cell was between the yolk and the mesoderm.

Embryos 5 mm. Long.

The cœlomic cavity is completely formed. With the growth in length a greater differentiation in tissues has been going on. The germ cells are found in the region of the hind gut, the majority of them are posterior to where the tail leaves the yolk. The most anterior cells are 0.080 to 0.220 mm. anterior to this region. The most posterior cells were 0.180 to 0.290 mm. anterior to the anus. With few exceptions the germ cells were found in the splanchnopleure (Fig. 3). In one embryo a nest of four cells was found in the somatopleure (Fig. 4). In the same embryo a germ cell was found lying in the center of the cavity between the somatopleure and the ectoderm. This was such an unusual position that it seems reasonable to think that it might have been torn away from the somatopleure in sectioning; however, the section has not the appearance of having been torn in any way. The size and appearance of the germ cells of embryos 5 mm. long are the same as in the preceding stage, but they are much more conspicuous due to the greater differentiation of the surrounding mesoderm. The cells are in the dorsal half of the splanchnopleure and often give the appearance of crowding toward the mesentery next to the alimentary tract (Fig. 5). The germ cells are distributed throughout a distance averaging 0.420 mm. in length. The following table shows in three embryos the unsymmetrical arrangement, the number of cells, the length of region throughout which the germ cells are distributed, the distance anterior to the anus of the most posterior germ cell and the distance anterior to the region where the tail leaves the yolk

	R.	L.	Total.	Length of Germ Cell Region, mm.	Dis. Ant. to Anus, mm.	Dis. Ant. to Tail, mm.
1.	6	26	32	0.420	0.100	0.230
2.	6	17	25	0.450	0.180	0.100
3.	9	7	22	0.400	0.290	0.220

of the most anterior germ cell. In embryo number 2, 2 germ cells were in the mesentery; 6 cells were in the mesentery in number 3.

Embryos 7 mm. Long.

Three embryos of this length were sectioned. In one of these there were 26 germ cells most of which were in the mesentery or at least very near it (Fig. 6). In the other two embryos the germ cells had probably divided once although there was little difference in the size of the cells from those in earlier stages. The cells were 14–16 microns in diameter and the nuclei 7–8 microns. There were 57 cells in one embryo and 63 in the other. In both embryos most of the germ cells were crowded in the mesentery in nests of 3 to 8 (Fig. 7). A few single cells were nearer the germ gland anlage or still in the splanchnopleure. Except in the case of one cell dividing, the appearance of the nuclei, cytoplasm, etc., seemed the same as in cells in earlier stages. The germ cells are in the same region as in the 5 mm. stage. The following table gives the number of cells, the length throughout which the cells were distributed, the distance anterior to the anus of the most posterior cell, and the distance anterior to the tail region of the most anterior cell.

	Total No.	Length of Region, Mm.	Dis. from Anus, Mm.	Dis. from Tail, Mm.
1.	26	0.680	0.120	0.220
2.	63	0.400	0.210	0.240
3.	57	0.430	0.120	0.320

Embryos 9 mm. Long.

In embryos of this length the anlagen of the germ glands appear as a fold in the peritoneum on either side of the base of the mesentery. (Figs. 8 and 9). This fold extends from 0.030 to 0.280 mm. anterior to the most anterior germ cell. In one embryo the fold was poorly developed for a distance of 0.080 mm. where there were no germ cells but this is not the most common condition. After the fold once appears, it is continuous throughout the region in which the germ cells are found. In one embryo there were no germ cells for a distance of 0.200 mm. but in this entire distance the fold of the peritoneum was very prominent. The fold seems to extend as far posterior from the germ cell region. It was found to extend 0.080 to 0.230 mm. posterior from the most posterior cell. In all of the embryos of this stage which

were sectioned the germ cells had found their way into the peritoneal fold. The number remains the same—28 to 30. The size and appearance of the cells agree exactly with the same in the earliest stages. The germ cells are distributed throughout a distance of 0.980 to 1.020 mm. The most posterior cells are 0.420 to 0.520 mm. anterior to the anus. It will be seen that although the embryos have increased much in size, the length throughout which the germ cells are distributed in proportion to the length of the embryo is about the same.

Embryos 14 mm. Long.

In all of the embryos of this stage the germ cells had divided at least twice. There had probably been two divisions in one embryo where the number was 144. Here the germ cells measure 8–12 microns in diameter and the nuclei are 4–6 microns. The chromatin is no longer in such fine granules as in the early stages but is often aggregated into small masses. The germ cells are in the center in the peritoneal fold with strands of peritoneal cells between them (Fig. 10). There is nothing which would suggest a differentiation of peritoneal cells into germ cells. Some of the germ cells are smaller than others but it is believed that these are cells which have divided more times than the larger ones and not that they are transition cells (see Fig. 10). No germ cells were found as large as even the smallest of those in younger stages. The germ cells and peritoneal cells stain as distinctly different as described before and no cells intermediate in size or staining qualities were found. In this embryo of 144 germ cells the germ gland or fold extends only 0.010 mm. anterior to the first germ cell but 0.500 mm. posterior from the most posterior germ cell. The germ cells were distributed throughout a distance of 1.280 mm. and the germinal fold throughout 1.790 mm.

In another embryo of the same length the germ cells had evidently divided several times—the cells here were only 6 or 7 microns in diameter and the nuclei about 34 in diameter. There are twenty or more germ cells in every section of each of the glands. The cells were not counted in the entire embryo. They are distributed throughout a distance of 1.220 mm. The most

posterior germ cell is 0.040 mm. posterior to the anus and the germ gland extends 0.160 mm. posterior to the anus. The germinal fold extends only 0.050 mm. anterior to the first germ cell.

Conclusions.

Germ cells in *Amiurus nebulosus* are distinct from all other cells at least from the time the embryo is 3.2 mm. long. They are then in the lateral plate of the mesoderm. The number of germ cells varies in the different embryos. Until the time when these cells are in or near the germ gland anlage, the average number is 23. With respect to the number of germ cells found on each side the embryo is not symmetrical. When the cœlome is formed they are largely in the splanchnopleure in which they migrate toward the mesentery and in this to the germ gland anlage. There is in most cases no multiplication in number of cells up to the time the germinal fold is formed. The cells migrate into this fold and then divide. There was no transition of peritoneal cells into germ cells observed in any of the stages studied. The epithelial covering and the stroma of the germ gland is derived from peritoneal cells. The germinal fold develops in regions where there are no germ cells.

This work was done at the university of Wisconsin under the direction of Prof. B. M. Allen, to whom the writer is greatly indebted for the material and for helpful advice and suggestions.

LITERATURE CITED.

Böhi, U.

'04 Beiträge zur Entwickelungsgeschichte der Leibeshöhle und der Genitalanlage bei den Salmoniden. Morph. Jahrb., 32, 1904.

Dodds, Gideon S.

'10 Segregation of the Germ Cells of the Teleost, Lophius. Journ. Morph., 21: 563–595. 1910.

Eigenmann, C. H.

'92 On the Precocious Segregation of the Sex Cells in Micrometrus aggregatus. Journ. Mroph., 5: 481–493. 1892.

'96 Sex Differentiation of the Viviparous Teleost Cymatogaster. Arch. f. Entwickelungsmechanick, 4: 125–179. 1896.

Hoffmann, C. K.

'86 Zur Entwickelungsgeschichte der Urogenitalorgane bei den Anamnia. Zeitschr. f. wiss. Zool., 44: 570–644. 1886.

MacLeod, J.

'81 Recherches sur la structure et le developpement de l'appareil reproducteur femelle des Téléostines. Arch. de Biol., 2, 1881.

Nussbaum, M.

'80 Zur Differenzierung des Geschlects im Thierreich. Arch. f. mikr. Anat., 18. 1: 121. 1880.

EXPLANATIONS OF THE FIGURES.

All figures were outlined with the aid of a camera lucida.

PLATE I.

FIGS. 1 AND 2. Germ cells in lateral part of mesoderm. × 390.
FIG. 3. Formation of cœlome. Germ cells in splanchnic layer. × 390.
FIG. 4. A nest of germ cells in somatopleure. × 390.
FIG. 5. Germ cells migrating toward mesentery. × 390.

BACHMANN.

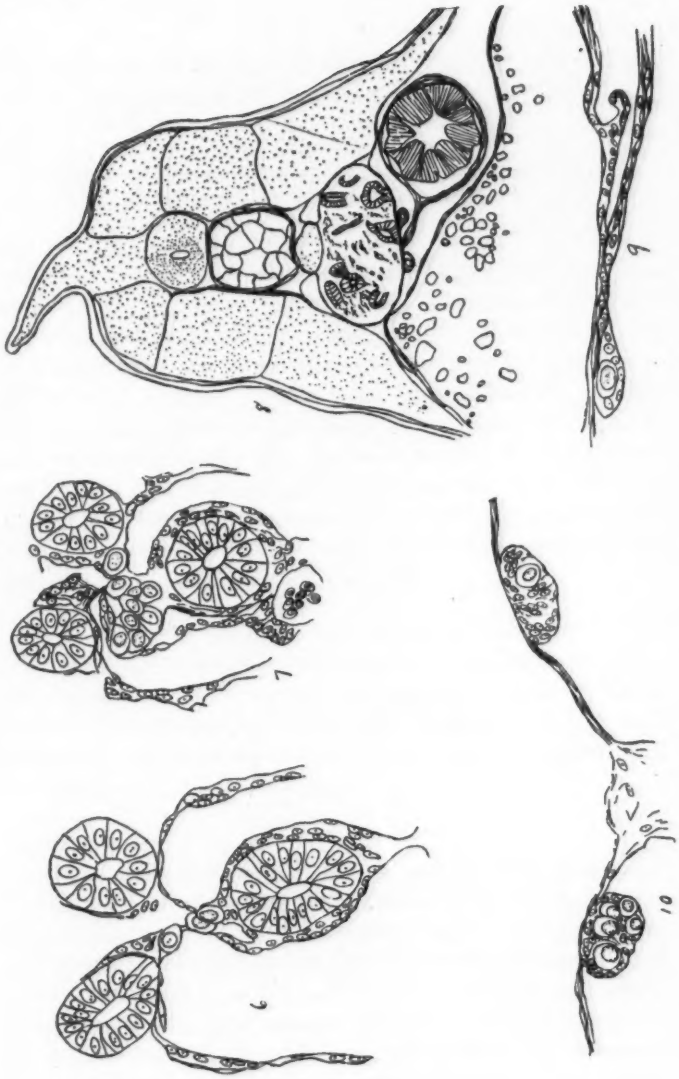
PLATE II.

FIGS. 6 AND 7. Germ cells migrating dorsally in mesentery. × 390.

FIG. 8. Diagrammatic representation of cross section of embryo to show the relative position of the germ glands. × 72.

FIG. 9. The origin of the germ gland from a fold in the peritoneum. × 390.

FIG. 10. Germ cells within the germ glands. × 390.



BACHMANN.

A QUALITATIVE ANALYSIS OF THE EGG-SECRETIONS AND EXTRACTS OF ARBACIA AND ASTERIAS.

OTTO GLASER.

(From the Marine Biological Laboratory at Woods Hole and the Zoölogical Laboratory of the University of Michigan.)

INTRODUCTION.

Although it must have been seen earlier, F. R. Lillie ('12) was the first, so far as I know, to throw emphasis on the observation that when unfertilized *Arbacia* eggs are allowed to stand in a small quantity of sea water, the supernatant liquid takes on a reddish-brown color. The amount of discoloration varies directly as the number of eggs present, the amount of sea water used, and within limits, the time of exposure. If the eggs are now precipitated with the centrifuge, and the fluid above is decanted and filtered through paper, or simply decanted without filtration, it can be very easily shown that substances are present which do not occur in ordinary sea water.

I. ANALYSIS BY MEANS OF SPERM-SUSPENSIONS.

The best method of analysis is that discovered by F. R. Lillie, and although it was not my primary purpose, I have verified a number of his results ('12[1], '13[1], '13[2]). Following Lillie, I shall present my material under four heads: (*A*) Activation; (*B*) Chemotaxis; (*C*) Agglutination; (*D*) Paralysis.

A. ACTIVATION.

1. *Iso-Activation.*—As Lillie has pointed out, the phenomena of activation are presented with unequal clearness in different forms. The spermatozoa of *Nereis* for instance are so active in sea water that the effect of the egg-secretion is obscured. With *Arbacia*, the sperm, as is well known, are also active in sea-water, nevertheless a noticeable increase in the rate of their movements can be observed after the addition of a drop of the egg-secretion. *Asterias forbesii* is really much more favorable than either *Nereis* or *Arbacia* for the detection of the activating effect, for here the

spermatozoa are almost motionless. The suspensions I worked with showed only isolated spermatozoa executing occasional spasmodic movements. Upon the addition of the secretion from its corresponding egg, the *Asterias* sperm are thrown into violent activity contrasting sharply with their original state of quiescence.

2. *Hetero-Activation.*—Essentially the same phenomenon described under the head of iso-activation can be observed if *Asterias* sperm are treated with *Arbacia* secretion, and *Arbacia* sperm with that from *Asterias* eggs. As might be expected the phenomenon is more marked with the *Asterias* sperm on account of their original inactivity, although the *Arbacia* sperm are also noticeably accelerated. I have made no experiments capable of deciding whether the activation of the two kinds of spermatozoa is due to the existence in the secretions from the two kinds of eggs of the same substance or of specifically different substances.

3. *Re-Activation.*—Activation is a temporary state, and after certain other reactions have occurred, the spermatozoa are found to be quite immotile. Such spermatozoa, although chemically different ([13]) from fresh ones which have never been subjected to the secretions, are nevertheless capable of re-activation. This is shown by the following experiments, in which *Arbacia* sperm-suspensions prepared in each case from a single male were divided into lots, activated by the addition of secretion, observed at intervals, and treated with more secretion as well as fresh eggs. The degrees of activation, reactivation, or movement are given as great, moderate, slight, or zero. In the instances in which eggs were added, fertilizations always took place, but the proportion of eggs that divided varied inversely with the length of exposure of the sperm to the secretion (p. 369).

B. Chemotaxis.

The chemotactic effect of the egg-secretion has been studied very carefully by Lillie, and both methods and results have been described by him at length ('[13]). I have verified the most essential results on *Arbacia* and have extended them to *Asterias*. The injected-drop method as well as the distribution of sperm about groups of eggs were used as indicators. As Lillie suggests, such results do not make clear the rôle of chemotaxis in normal

ARBACIA SPERM.

Experiment 1.

Treatment.	Lot A_1. Activation.	Lot A_{11}. Activation.	Lot A_{12}. Activation.	Lot A_{13}. Activation.	Lot A_{14}. Activation.	Time.
Added secretion	Strong	Strong	Strong	Strong	Strong	11.05 A.M.
	Moderate	Moderate	Moderate	Moderate	Moderate	11.10
Added secretion	.....	Strong	.....	.....	.....	11.10
Added eggs....	.....	.....	Strong	.....	.....	11.10
	.....	Slight	Slight	.....	.....	11.11
	Zero	.....	.....	Zero	Zero	3.30 P.M.
Added secretion	.....	.....	.....	Slight	.....	3.30
Added eggs....	.....	.....	.....	.....	Slight	3.30

Experiment 2.

Added secretion	Strong	Strong	Strong	.....	.....	12.10 P.M.
	Slight	Slight	Slight	.....	.....	1.10
Added secretion	.....	Moderate	.....	.....	.....	1.10
Added eggs....	.....	.....	Moderate	.....	.....	1.10

Experiment 3.

Added secretion	Strong	Strong	Strong	Strong	Strong	12.00 M.
	Slight	Slight	Slight	Slight	Slight	1.00 P.M.
Added secretion	.....	Moderate	.....	.....	.....	1.00
Added eggs....	.....	.....	Moderate	.....	.....	1.00
	.....	.....	.....	Zero	Zero	5.00
Added secretion	.....	.....	.....	Slight	.....	5.00
Added eggs....	.....	.....	.....	.....	Slight	5.00

fertilization. One of the difficulties is the thigmotactic response of the sperm, as this insures their sticking to surfaces they may chance to meet. It is easy to see how under such circumstances an accumulation about eggs might take place. While not conclusive, experiments in which the surface of the eggs was very materially reduced, and the jelly mass quite obliterated by desiccation, showed that even in this case the sperm collect in great numbers about the eggs. Minute fragments of egg-powder are also centers about which the sperm aggregate in great numbers. I have not observed this when powdered glass or sand were added to the suspensions and as the surfaces in these cases although of different physico-chemical properties, must be assumed to be at least as extensive as in the egg-powder, the marked sperm aggregation about the dried egg-fragments and its absence about the other fragments is certainly in harmony with the idea that chemotaxis is a factor in bringing the sperm to the egg.

C. Agglutination.

1. *Iso-agglutination.*—That egg-secretion is capable of agglutinating sperm has been fully described by Lillie ('12[1], '13[1], '13[2]). In this section I simply wish to add my testimony to his as to the facts in the case—namely that this phenomenon occurs; that it is reversible; that its duration is brief and may be utilized as a measure of concentration; and that sperm once agglutinated, although capable of re-activation, do not agglutinate again.

2. *Hetero-agglutination.*—I have found that the *Arbacia* secretion also agglutinates the sperm of *Asterias*, and that *Asterias* secretion, besides agglutinating its own, has a similar effect on the spermatozoa of *Arbacia*. Thus

1. *Arbacia* sperm + *Arbacia* secretion = agglutination masses described by Lillie.
2. *Arbacia* sperm + *Asterias* secretion = dense, angular masses. Reaction slower than 1.
3. *Asterias* sperm + *Asterias* secretion = masses smaller than 1. Angular.
4. *Asterias* sperm + *Arbacia* secretion = result similar to 3.

At the time when these experiments were performed, the material had practically disappeared, and in consequence the question whether the above hetero-agglutinations are due to the same substances as the iso-agglutinations, or whether each secretion contains both an iso- as well as a hetero-agglutinin, could not be decided. Since hetero-agglutination between *Arbacia* and *Nereis* is not brought about by the same substance ('13[1]) that causes the iso-agglutination, the same relations may hold for *Arbacia* and *Asterias*. It will prove interesting to see whether the agglutinin in *Arbacia* secretion that reacts with the *Nereis* sperm is the same one that agglutinates *Asterias* spermatozoa. If not, it becomes important to discover how many agglutinins are present. In this connection I may refer to an observation already published (13[3]), namely that the *Arbacia* secretion agglutinates the larvæ of *Arenicola*.

3. *Origin of the Agglutinin.*—Lillie has shown that the agglutinin is chiefly located in the outer jelly of the *Arbacia* egg. I have found the same thing to hold true of the *Asterias* ovum. It was also proved by Lillie (13[2]) that if the outer jelly is removed

by shaking, the eggs after two or three washings impart only a weak agglutinating power to the supernatant water. This however increases in the course of time. From this Lillie concludes that the eggs produce the agglutinating substance. Moreover he has shown that they are the only tissue of *Arbacia* that does produce this material.

My own experiments show that the agglutinating substance is located in greatest abundance in the jelly and that the eggs also contain this material. As additional evidence it may be stated that when eggs are inseminated with fairly concentrated sperm-suspensions, the collection and activity of the spermatozoa may be great enough to tear the outer jelly away from the egg. When this occurs, one may suddenly observe great balls of sperm apparently cast off from the eggs and forming huge agglutination masses. In this instance many sperm also remain in contact with the egg, which later shows a typical fertilization membrane, and divides. This point is important in connection with the mistaken idea that the outer jelly is essential for the appearance of the fertilization membrane ('13[4]).

In connection with the existence of agglutinin in the egg, I may refer to experiments with egg-powder in which very effective agglutinations were secured. It cannot well be supposed that every fragment of egg-powder has bits of dried jelly adhering to it.

4. *The Mechanism of Agglutination.*—On this point Lillie's inference based on the *Nereis* sperm, in which because of relatively great size and slow movement, direct observation is possible, can be substantiated by a variety of observations. Lillie noticed that the agglutination is between the heads, and that the tails, at least until the period of paralysis sets in, are not visibly affected. "The adhesion of the heads demonstrates some change in the membrane that renders them sticky" ('13[1], p. 556), and direct observation showed "that in agglutinated masses the heads of many of the spermatozoa are swollen into spherical form and have lost the normal strong refringibility. The change is in this case a very characteristic one indicating a great change in permeability" (*loc. cit.*).

The capacity for influencing the permeability of cells is by

no means limited to the sperm. Thus, as I have pointed out ('13[3]), egg-extract added to blastulæ which have developed in normal sea-water, slows their movements, and increases their volume, indicating a change in permeability. R. S. Lillie has emphasized on numerous occasions the great advantages of *Arenicola* embryos ('13[5] and earlier papers) as indicators of permeability changes which may be registered by the outflow of pigment. When the *Arbacia* secretion is added to the *Arenicola* larvæ, movement is slowed down, the pigment flows freely into the water, indicating an increase in permeability, and a slight and reversible agglutination occurs. Considering all the facts at present available, it seems reasonable to suppose that agglutination is the result of an increase in permeability, and we may imagine that the exudation of material from the cells or the changes that lead to the exudation, render them sticky. Unless some other chemical reaction is involved, it seems to me more likely that the occurrence of agglutination depends upon the exudate. The sperm of *Nereis*, the blastula of *Arbacia*, and the larvæ of *Arenicola* appear to furnish us with three out of four theoretically possible types of cases.

D. Paralysis.

The addition of egg-secretion to a sperm-suspension is followed by activation, a chemotactic effect, and a reversible agglutination. For some time after the agglutination masses have disappeared, the sperm remain quite active, but the rate of their movements decreases until finally they come to a standstill, and appear as though paralyzed. As the re-activations show, this paralysis is not an irreversible state, although the second period of activity never lasts as long as the first, nor is the activity on the whole as great while it lasts. The third activation may be almost momentary. Re-activated sperm are capable of fertilizing the eggs.

II. QUALITATIVE CHEMICAL ANALYSIS OF THE EGG-SECRETION.

Although the observations recorded are important for an understanding of the nature of the egg-secretion, they have been reported at this time and in this connection chiefly for the interest they may have in relation to the factual basis of Lillie's

theory of fertilization ('13[2]). Analyses by other methods were attempted.

It is altogether likely, and in the case of the *Nereis-Arbacia* hetero-agglutination, definitely proved, that the egg-secretion contains more than one substance. The problem therefore presents itself of isolating these bodies. A first step toward orientation has been made by means of certain qualitative chemical tests.

The method of securing egg-secretion finally adopted was suggested to me personally by Dr. F. R. Lillie, and consists in adding to a certain number of "dry" eggs, double their volume of sea-water, and with occasional slight agitation, allowing ten minutes to elapse. At the end of this time the ova were precipitated by 100 revolutions of the centrifuge, and the supernatant fluid, a clear, golden liquid in the case of *Arbacia*, or whitish and opalescent in the case of *Asterias*, was usually carefully decanted without filtration through paper. Such solutions I have adopted as standard. With the *Arbacia* secretion the following tests were made:

1. The solution is gold-yellow in color and clear.
2. The solution is neutral to litmus.
3. Upon cooling to 0° no change was noticeable.
4. Upon boiling the color becomes faintly purple.
5. The purple coloring matter may be removed at least in part if white of egg is allowed to coagulate in the boiling solution.
6. No acid-insoluble precipitate is formed upon the addition of $n/10$ NaOH.
7. 1 or 2 drops conc. HCl produced faint cloudiness which became more distinct on standing.
8. The addition of alcohol produced no visible change.
9. Millon gave a white precipitate with no color change on boiling.
10. The biuret test was negative.
11. HNO_3 gave no ring but a faint cloudiness.
12. The xanthoproteic test gave no precipitate, but the solution turned distinctly yellow.
13. The Adamkiewicz test was negative.
14. Fehling gave no reduction.

15. Bi-subnitrate gave no reduction.

So far as these tests go, and they were repeated several times, it seems likely that reducing substances are absent, and that if proteins are present, their concentration is too low to give the ordinary reactions in their usual form. The opalescence observed upon the addition of acids, and the yellow color gotten in the xanthoproteic test, indicate possibly minute traces of protein but these may come from traces of the egg jelly.

With all the tests that made such experiments possible, the secretion was afterwards tested as to its agglutinating property, and was found in every case to still possess this power, tests 3, 4, and 5. No exact quantitative comparison as to the agglutinating strength before and after boiling was made. Lillie states ('13[1], p. 557) that the agglutinin when boiled and then allowed to stand at 95° for 30 minutes is destroyed in large part, and almost entirely if kept at this temperature for 66 minutes. In some of my experiments the secretion was brought to the boiling point, in others it was boiled 5 minutes. The color change noted always occurred, but the agglutination power appeared undiminished. Both of these results are described in Lillie's paper ('13[1]).

As to the nature of the purple substance, I may say that even if its formation, and presumably with that, the abstraction of something from the original solution makes no measurable difference in the agglutinating power of the secretion, this material may nevertheless be significant in other connections. My reasons for suggesting this are that when a sperm suspension is added to the secretion, traces of this purple color appear; when dilute sperm suspensions are killed by heat the same color is seen; in concentrated suspensions the red dominates over the blue; and in still more concentrated suspensions the color is like that of port wine. This same color also appears in desiccated eggs as well as sperm. From these facts the thought lies near at hand, that we are here dealing with the production of a compound specific for *Arbacia*. Corresponding experiments with *Asterias* do not give this color, nor have I gotten it with oyster sperm. On the other hand, *Asterias* sperm as well as egg-secretion turn a slight salmon-color when boiled. With the exception of these

color differences, the same results in tests, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, and 14, were gotten with the *Asterias* secretion.

III. THE SECRETION OF SUBSTANCES AT FERTILIZATION AND IN HYPERTONIC SEA-WATER.

The unfertilized eggs of *Arbacia* show no noticeable decrease in volume in sea-water even four hours after removal from the ovaries ('14[1]). Fertilized eggs however are measurably smaller in both *Arbacia* and *Asterias* within a few minutes after insemination ('13[2], '14[1]). A change in the same sense seems also to occur in the lamprey ('14[2]).

The decreased volume is probably chiefly due to the loss of water but it can be shown that in *Arbacia* other substances also leave the egg.[1]

The first thing that occurs to one in testing whether at fertilization more soluble substances are secreted than from unfertilized eggs, is to compare the agglutination strength of equal quantities of sea water, with and without sperm, to which equal quantities of unfertilized eggs have been added and allowed to remain for equal lengths of time. Such experiments show that there is more free agglutinin present in the sterile water. The reason for this is not necessarily that the eggs at fertilization secrete less of this substance, for Lillie has shown that the sperm binds the agglutinin, a fact also indicated by the experiments on re-activation without re-agglutination. Moreover it is quite possible that at fertilization other substances are secreted and combine with the agglutinin outside the egg. This alone or in conjunction with the circumstance that a portion of the agglutinin in these experiments would already be bound by the sperm in the infected sea-water would account for the finding of a smaller amount of free agglutinin by any subsequent agglutination test.

[1] McClendon ('09) says of *Arbacia:* "When the egg is fertilized or put in 'membrane-forming' solutions a fluid is extruded which pushes the jelly out from the surface of the egg." As neither the evidence for this statement, nor any reference to the fact that something is also extruded from the unfertilized eggs, are given, I do not know whether my contention is antedated or not. McClendon also states that parthenogenetic reagents, when sufficiently concentrated cause the diffusion of pigment from the eggs into the sea-water. If these concentrations are identical with those most favorable for the initiation of cleavage, there is here an important point of identity between the artificial and normal induction of development.

Such experiments therefore are not capable of deciding whether more or less soluble substance is secreted at fertilization than before. That no agglutinin is secreted after fertilization is complete, has been demonstrated by Lillie's ('13[2]) later work.

The second possibility, that of utilizing the xanthoproteic test suggested itself, for in case the yellow color were not due to traces of dissolved jelly, it might increase as the result of fertilization. As a matter of fact the yellow color gotten from sea-water over eggs inseminated as above is deeper than that given by an equal quantity of sterile water exposed to an equal quantity of unfertilized eggs for the same length of time. This was considered a promising result until it was found that the addition of even a small quantity of sperm increases the density of the color to a marked degree.

A third test however gave comparative results which are not open to this difficulty. In these experiments I made use of the fact that the unfertilized eggs of *Arbacia* discolor the sea-water. Three sets of observations were made. In each of these 1 c.c. of an egg-suspension of given concentration in which the ova were uniformly distributed, was added to each of three tubes, one of which contained 5 c.c. of sterile sea-water, the second 5 c.c. of sea-water infected with just enough spermatozoa to be very faintly opalescent, whereas the third contained 5 c.c. of "double" sea-water, *i. e.*, sea-water which had been boiled down to half its original volume. Color tests were made in the usual way, and the discoloration produced by the unfertilized eggs in a given time was taken as unity. On this basis the following results were obtained in an experiment exactly representative of the others:

	A	*B*	*C*
Time.	5 C.c. Sea-water, 1 C.c. Eggs.	5 C.c. Infected Sea-water, 1 C.c. Eggs.	5 C.c. Double Sea-water, 1 C.c. Eggs.
9.40	Color = 0	Color = 0	Color = 0
9.50	Color = 1	Color = 1.5	Color = 2
9.55	Color = 1	Color = 1.5	Color = 2
9.56	+ 1 c.c. eggs	+ 1 c.c. eggs	+ 1 c.c. eggs
10.10	Color = 2	Color = 3.0	Color = 4

The extra c.c. of eggs was added at 9.56 to see whether the first difference between *A* and *B* could be the result of either the slight

opalescence due to the presence of the small amount of sperm in *B*, or to the small traces of the purple "sperm-agglutinin" compound which was necessarily formed in this tube. However the relative densities of the colors in the three tubes did not change, although the relative effect of the sperm as well as of the purple compound could not have been as great at 10.10 as at 9.50, and 9.55. The same experiment was repeated several times with more dilute suspensions of sperm, and very dense suspensions of eggs. Exactly the same relative discolorations were obtained. I mixed up the tubes on several occasions and asked some one not familiar with the experiment to see if any differences could be noted. I also had the tubes mixed and handed to me for identification. In every case it took but a moment to distinguish the tube in which fertilization had occurred. This was true also in the absence of the tube containing the double sea-water.

One may say therefore that when equal quantities of unfertilized *Arbacia* eggs are allowed to stand for equal lengths of time in equal quantities of sterile and sperm-infected sea-water, the discoloration of the supernatant liquid is greater in the case of the eggs undergoing fertilization. This proves that something in addition to water leaves the eggs at fertilization, a circumstance not at all surprising in view of the fact that, whatever else it may involve, fertilization is accompanied by an increase in permeability.[1] The question whether there is in addition to the quantitative difference, also a qualitative one, must for the present remain open.

[1] Loeb ('13[4]) criticizes McClendon's evidence for increased permeability after fertilization on the ground that more than one interpretation of the experimental evidence is possible. My contention is not that the permeability of fertilized eggs is greater than that of unfertilized, but that there is an increase in the permeability of unfertilized eggs at the moment at which they are being fertilized. This idea is expressed by R. S. Lillie, p. 290, "The Physiology of Cell Division," III. Direct observational support for this view is furnished by F. R. Lillie's work on *Nereis*, and more indirect evidence by my measurements of the rate of secretion by unfertilized eggs as compared with eggs undergoing the process of fertilization. The decreased volume of the *Arbacia* and *Asterias* ovum after fertilization seems to me unintelligible except as the result of an increase in permeability. Why it should be assumed that this increase is more than momentary, I fail to see.

IV. ANALYSIS BY MEANS OF THE RATE OF CLEAVAGE.

A. The Effect of Egg-Secretion in Normal and Alkaline Sea-Water.

A first step toward an answer however has been taken in the form of experiments in which known quantities of egg-secretion were added to normally fertilized eggs and the rate of cleavage compared with controls. The secretions used in these experiments were standard, prepared as before.

Many notes were taken in making these comparisons for it could not be foreseen which details might be utilized as indicators of the relative rates of development. Of all these however only certain data with respect to the 2, 4, and 8-cell stages are reported. This choice depends entirely on the advantage of reporting the more easily verifiable facts, and not because other details are in any way contradictory. Indeed the reverse is true and applies to such stages of the division spindles as can be clearly recognized in the living egg. Similar conclusions also can be drawn from the later stages, although it is much more difficult to tell at a glance whether a certain culture of blastulæ, gastrulæ or plutei, is more or less advanced than a given standard. I wish to emphasize the fact however that these later stages were obtained in large yields in the experimental cultures and were normal, though often somewhat slower of movement.

Several ways of comparing the rates of cleavage are open. Of these the following two were adopted: the time (minimum, in the table) that elapsed between insemination and the first 2, 4, or 8-cell stage seen in a particular culture is given, as well as the interval (maximum, in the table), between insemination and the time at which the cultures were at the height of these respective stages. The results of the second way of counting are in the same sense as the others, but are less accurate since there is greater opportunity for errors of judgment, whereas no error of judgment is likely with respect to the recognition of the first 2, 4, or 8-cell stage seen. The possibility that the particular cases observed were not the first to appear in the cultures of course remains, but a moment's examination of Table I will show that with very considerable errors of this sort consistent results could hardly have been obtained.

The observations were so made that intervals of one minute elapsed between the readings. Thus if *A*, *B*, *C*, *D* represent four culture dishes, *B* was read one minute after *A*, *C*, one minute after *B*, *D* one minute after *C*, and the second reading of *A* was taken four minutes after the first. Under such conditions one could introduce quite a large error from the mere arrangement of the dishes. This was guarded against both by reversing the order of reading from time to time, as well as by changing the order of the dishes in the several experiments. As a matter of fact the significant differences in my tables are always greater than the maximum error which might have resulted from the order of observation.

In the presentation of the results I have not compared the intervals for each of the three stages considered, separately (although the data for such comparison are given), but the average of the intervals that elapsed between insemination and the 2, 4, and 8-cell stages respectively. This procedure further eliminates errors attaching to any specific observation, besides reducing the number of comparisons necessary.

In the right hand division of the following table are contained comparable observations made in sea-water whose C_{OH} had been raised by the addition of 1.75 c.c. *N*/10 NaOH to 100 c.c. sea water. These experiments are included because they serve as checks on those in normal sea water. The theoretical considerations which prompted them were based on the fact brought forward by Loeb ('98) that the development of *Arbacia* is accelerated in alkaline sea-water, and depressed in acid. This is easily verified if normally fertilized eggs are allowed to develop to the blastula stage and are then divided into three lots, one for control, a second to which NaOH is added in the proportion of 1.75 c.c. *N*/10 per 100 c.c. of sea water, whereas the third is acidulated with HCl in the proportion of 1.75 c.c. *N*/10 per 100 c.c. of sea water. In such an experiment gastrulæ, with only here and there a short armed pluteus, predominate in the HCl culture at a given hour; the control at the same time contains a large number of plutei in various stages and some late gastrulæ, whereas the NaOH dish holds practically nothing but complete plutei. Loeb, in the paper referred to attributed this result to

the probable acceleration of the oxidations due to the increase in OH ions. I used the NaOH in conjunction with the egg secretion on the theory that if the secretion affects the oxidations it should antagonize the effect of the NaOH, since the secretion alone, in the concentrations employed, retards the development. As measured by the first three cleavages however, the effects are not antagonistic, but additive, for the NaOH if anything had an effect exactly the reverse of the expected and that actually found for the later stages. This result is in harmony with Loeb's later work. In his recent book ('13[8]), p. 35, he says: "The writer published years ago a paper in which he showed that the development of the eggs of *Arbacia* is retarded and finally inhibited if increasing quantities of acid are added to the sea water. He has since vainly attempted to show that the rate of development of the sea urchin egg can be increased with the increase of the concentration of hydroxylions in the sea water." If the reference here is to the paper cited above, evidence is there given that the increase in hydroyxlions does accelerate the development of *Arbacia*, but this acceleration was not noticed clearly until the day after fertilization. Both results seem really to be correct, only the rate of cleavage is either not accelerated or even depressed, whereas the rate of development from the blastula to the pluteus, is accelerated.

TABLE I.

RATES OF CLEAVAGE IN MINUTES AFTER INSEMINATION.

Cells.	Sea-Water 2 Vols.		Sea-Water 1 Vol. Secretion 1 Vol.		NaOH Sea-Water 2 Vols.		NaOH Sea-Water 1 Vol. Secretion, 1 Vol.		
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	
2	44′	53′	47′	59′	45′	52′	54′	77′	Experiment I
4	64	74	73	92	52	74	72	93	
8	83	91	.....	.....	74	95	.....	.....	
2	48	52	47	70	53	80	51	70	Experiment II
4	48	71	55	83	80	.....	70	88	
8	71	84	63	88	85	.....	88	.....	
2	39	53	41	66	40	46	41	64	Experiment III
4	42	74	66	91	46	76	.....	77	
8	.....	92	95	.....	82	93	94	.....	
2	45	67	50	74	42	59	61	79	Experiment IV
4	48	72	50	88	44	86	73	109	
8	72	107	98	113	70	98	.....	.....	
Average rate....	55	74	62	82	59	76	76	for first three divisions	
Average rate....	47	65	54	78	50	66	64	82 for first two divisions	

It is apparent from these figures that whether we base our averages on the time that elapses between insemination and the minimum number of a given stage or the maximum, the results are always in the same sense. The special calculations based on the 2 and 4-cell stages have been made for all the columns in order to make fair a comparison of the first three with the last in which the records do not include the 8-cell stage. It seems reasonable to assert that the presence of egg-secretion in certain concentrations retards the early development of the *Arbacia* ovum, and that the presence of NaOH in definite concentrations increases the retardation.

B. The Effect of Egg-Extract in Sea-Water.

Experiments with egg-extract, corresponding to those with egg-secretion were made for the purpose of determining whether the egg contains, and is therefore possibly able to secrete during the brief period of increased permeability incident to fertilization, substances whose effect on the rate of development might be different from that of the secretions heretofore used. Extracts were made by the use of hypertonic sea-water ("double sea-water"), by grinding the eggs with pulverized glass as well as by laking them in distilled water. The quantities and concentrations used corresponded as nearly as possible to the amounts of secretion employed in the earlier experiments although other concentrations were tried. In all cases marked retardation of development was observed. Owing to the fact that the extracts prepared by the three methods are not identical and that their analysis has not been carried far enough as yet, accurate comparisons with the secretion are at this time impossible. However three characteristics of extract-cultures contrasted sharply with cultures developing in the presence of secretion. These were: numerous arrests of development in the early cleavages; much cytolysis; and a very general failure to get beyond the early non-motile blastula. These results indicate a qualitative difference between the secretion and the extract.

V. THEORETICAL.

The heightened rate of oxidation, the increased rate of secretion together with the decrease in volume on impregnation, all suggest

as the essential point in the initiation of development, the relief of antagonistic conditions. When I found that the egg-secretion in certain concentrations actually retards development, I thought I had located the antagonists, and, without knowledge of the earlier and similar suggestion by R. S. Lillie ('09), postulated that initiatory agents are effective "because through increased permeability of the plasma film the egg is enabled to loose substances antagonistic to oxidation" ('13[3], p. 450).

It would of course have been more conservative to say "antagonistic to development," for the retardation brought about by the secretions and extracts may be the outcome of interferences with other conditions and processes, no less essential for normal development, than the oxidations. Indeed Loeb and Wasteneys ('11) have demonstrated the independence of the temperature coefficients of oxidation and cleavage, so that retardation of the latter is not synonymous with a depression of the former. Again the secretion, in the concentrations in which it was employed, brings about abnormal permeability relations which might account for the retarded development, and the possibility that the substances involved have one effect in the concentrations in which they occur in the unfertilized egg, but different effects in the higher concentrations of the experiments, must not be overlooked for good analogies are to be found in the effects of different concentrations of ether (R. S. Lillie, '12[2], p. 373). While it may yet be true that the delaying effect of the secretion is actually the outcome of a depression in the rate of oxidation, proof of this must be sought in further experiments.

However, even if further experimentation should succeed in tracing the retarded development to a decrease in the oxidation rate brought on by the presence of the egg-exudate, a difficulty would still remain, for the surface film of the unfertilized ovum is permeable for the secretion, and so by constant elimination an accumulation of the suspected antagonist to the inhibition point would be automatically prevented. This is the argument applied by Loeb in contraversion of R. S. Lillie's idea that CO_2 might be the antagonist, for inasmuch as CO_2 "is a good agency for calling forth membrane formation" and as only substances capable of diffusing into the egg can have this effect, the egg surface

must be permeable to it before impregnation, and consequently there can be no accumulation that could be relieved as the result of the increase in permeability associated with fertilization.

However two possibilities remain; either the quantitative difference in the rate of secretion between unfertilized ova and ova undergoing fertilization is significant, or there is a qualitative difference as yet undetected between the secretions from the two kinds of eggs. Since "the velocity of segmentation in eggs fertilized by two spermatozoa is identical with that found in eggs fertilized by one. . . . ," we must believe, according to Loeb ('13[6], p. 13) "that the spermatozoan causes development . . . by removing an obstacle to development."

Certain facts and considerations to be dealt with in a later paper, seem to point to the possibility that in addition to the quantitative difference, the secretion at the moment of fertilization differs from the secretion of uninseminated eggs, qualitatively as well. Should further experimentation establish the correctness of this suspicion, then since the rate of oxidation in fertilized eggs is greater than in unfertilized, we could still say, "substances antagonistic to oxidation are eliminated at fertilization" although these substances may be neither CO_2 nor any of the constituents of the secretion from unfertilized eggs. Furthermore their action may be indirect through the inhibition of processes which when set going by their removal allow oxidation to proceed at the heightened rate normal for the fertilized egg. Whatever the final solution of these problems may be, it seems altogether likely that the initiation of development, and with it, the initiation of the processes leading to cell division, are in some way significantly related to the momentary increase in the permeability of the ovum, accompanying the process of fertilization.

VI. SUMMARY.

1. In corroboration of F. R. Lillie, it was found that the egg-secretions of *Arbacia punctulata* exert a chemotactic effect on sperm, and activate, agglutinate, and paralyze them.

2. The egg secretions of *Asterias forbesii* behave in a similar manner toward *Asterias* sperm.

3. *Arbacia* secretion activates, agglutinates, and paralyzes

Asterias sperm, and *Asterias* secretion has the same effects on *Arbacia* sperm.

4. Paralyzed sperm may be reactivated but not reagglutinated.

5. The egg secretions test negatively for reducing substances and do not give the usual protein tests although they were found to be faintly positive to the acid tests and the xanthoproteic. This may be due to traces of the egg jelly.

6. Agglutination may be gotten with dry egg powder.

7. The agglutination reaction very possibly depends on a surface effect.

8. More soluble substances escape from the egg of *Arbacia* in hypertonic sea-water and in sea-water infected with sperm, than from unfertilized eggs in normal sterile sea-water in the same length of time.

9. The egg-secretion in certain concentrations retards development measurably.

10. 1.75 c.c. $N/10$ NaOH added to 100 c.c. of sea water does not accelerate the early cleavages. The retardation noted may well be within the limits of error.

11. NaOH in the same concentration does markedly accelerate the development of blastulæ into plutei.

12. Egg secretion in the concentrations employed + NaOH in the concentration given above, results in a more marked retardation of cleavage than the egg-secretion without the NaOH.

13. Egg-extract, as contrasted with egg-secretion, in addition to retarding development, in a similar manner when employed in a similar manner, results in cytolysis, arrests of development in the early cleavages, and a general failure of the eggs to get beyond the early non-motile blastula.

14. The heightened rate of oxidation in the fertilized egg; the increased rate of secretion in eggs undergoing fertilization; the decreased volume after fertilization all point toward the possibility that initiation of development depends upon the removal of substances, directly or indirectly antagonistic to oxidation. Proof that the egg-secretion in certain concentrations measurably retards development is however insufficient evidence either for the conclusion that it itself is the antagonist, or contains it.

This would not follow even if it were shown that the retardation is the result of depressed oxidation. It is possible however that egg-secretion, at the moment of fertilization differs qualitatively from the earlier secretion, and contains the real antagonists whose inhibitory effect need not necessarily be thought of as having been direct.

15. These suggestions together with the facts upon which they are based, are not necessarily out of harmony with existing prominent theories of fertilization.

UNIVERSITY OF MICHIGAN,
December 20, 1913.

LITERATURE.

Loeb, Jacques.
'98 Über den Einfluss von Alkalien und Sauren auf die Embryonale Entwicklung und das Wachstum. Arch. f. Entwick. mech., 16.
Lillie, R. S.
'09 On the Connection between Changes of Permeability and Stimulation, and on the Significance of Changes in Permeability to Carbon Dioxide. Amer. Journ. Physiol., 24.
McClendon, J. F.
'09 On Artificial Parthenogenesis of the Sea-Urchin Egg. Science, XXX.
Lillie, R. S.
'11 The Physiology of Cell-Division. Amer. Journ. Physiol., XXVII.
Loeb and Wasteneys.
'11 Sind die Oxidationsvorgange die unabhangige Variable in den Lebenserscheinungen? Biochem. Zeitschr., XXXVI.
Lillie, F. R.
'12[1] The Production of Sperm Iso-Agglutinins by Ova. Science, XXXVI.
Lillie, R. S.
'12[2] Antagonism between Salts and Anaesthetics. Am. Journ. Physiol., Vol. 29.
Lillie, F. R.
'13[1] Studies of Fertilization V. Journ. Exp. Zool., 14.
'13[2] The Mechanism of Fertilization. Science, XXXVIII.
Glaser, Otto.
'13[3] On Inducing Parthenogenesis in the Sea Urchin (Arbacia punctulata) together with considerations on the Initiatory Effect of Fertilization. Science, XXXVIII.
Elder, J. C.
'13[4] The Relation of the Zona Pellucida to the Formation of the Fertilization Membrane. Arch. f. Entwick. mech., 36.
Lillie, R. S.
'13[5] The Rôle of Membranes in Cell-Processes. Pop. Sci. Mo., 1913.
Loeb, Jacques.
'13[6] Artificial Parthenogenesis and Fertilization. Chicago, 1913.

Glaser, Otto.

'14[1] The Change in Volume of Arbacia and Asterias Eggs at Fertilization. BIOL. BULL., Vol. XXVI.

Okkelberg, Peter.

'14[2] Volumetric Changes in the Egg of the Brook Lamprey after Fertilization. BIOL. BULL., Vol. XXVI.

Glaser, Otto.

'14[3] An Analysis of the Egg-Extractives of Arbacia and Asterias. Science, Vol. XXXIX.

ON AUTO-PARTHENOGENESIS IN ARBACIA AND ASTERIAS.

OTTO GLASER.

(From the Marine Biological Laboratory at Woods Hole and the Zoölogical Laboratory of the University of Michigan.)

The unfertilized ova of *Arbacia* eliminate substances which discolor the sea-water, but when the same eggs are undergoing impregnation, the discoloration of an equal amount of sea-water in unit time, is roughly half again as much ('14[2]). Hand in hand with this fact goes the discovery that both *Arbacia* (*punctulata*) and *Asterias* (*forbesii*) ova, are measurably smaller in volume after fertilization than before ('13[1], '14[1]). These observations led me to begin an investigation which might throw some light, not only on the nature of the substances lost, but on the further question whether their elimination is significantly associated with the initiation of development. Not more than a beginning has been made, but some of the preliminary, chiefly qualitative, results are sufficiently interesting to be reported at the present time.

My earliest experiments consisted in a series of attempts to find what sort of substances could be gotten out of the eggs. I therefore prepared solutions in sea-water from ground, laked, or extracted ova. These preparations when tested with sperm exhibited the properties which had been described by F. R. Lillie for egg-secretion ('12, '13[2,3]). Emphasis must be laid on the power of these extracts to activate the sperm; on their chemotactic effect; and on their sperm-agglutinating as well as sperm-paralyzing capacities. Addition of the extracts in certain concentrations to normally fertilized eggs, resulted in a retardation of development; normal blastulæ instantly slowed their movements, and underwent a noticeable increase in volume when subjected to the extracts. Similar observations were made on the larvæ of *Arenicola* whose rate of movement was also slowed down, to be followed instantly by an outflow of their yellow pigment and a slight and reversible agglutination.

From these preliminary observations it appeared that the egg-extracts are capable of increasing the permeability of cells. On the view therefore that an increase in permeability is associated, significantly or otherwise, with fertilization, the initiation of development by means of these extracts should be possible. Against this idea stood the evidence of Loeb ('11) according to which "it was found that it was absolutely impossible to cause membrane formation of the sea-urchin egg by extracts from the sea-urchins of the same or even related species." From this Loeb concluded that the cortical layer of the egg exhibits against the lysins of the body producing it, the immunity characteristic for genetically related cells in general. It seemed little likely therefore that extracts prepared from the cells themselves should result in the initiation of development, and as a matter of fact, "membrane formation" in the sea-urchin egg did not occur. On the other hand, in corresponding experiments on the eggs of *Asterias*, a typical fertilization membrane did appear, and in both sea urchin and starfish, development was successfully initiated. For the type of initiation here dealt with, I have chosen the term auto-parthenogenesis.

Auto-Parthenogenesis in *Arbacia* by Means of Egg-Secretion.

The solutions employed in these early experiments, despite the use of weighed quantities of eggs, were not only very variable in concentration, but also differed in composition according to the method of preparation ('14[2]). Since the secretion from unfertilized eggs, has the same effects on sperm as the extracts, it appeared possible that it might also have similar effects on blastulæ, *Arenicola* larvæ, and unfertilized ova. This indeed was soon found to be the case, although as pointed out elsewhere ('14[2]) extract and secretion are probably not identical throughout.

The next step, the discovery of a more reliable mode of procedure, resulted in a definite method of securing results, but I have no reason for considering it final. Omitting the experiments of orientation and other unnecessary details, the following outline may be taken as a guide for further work: Standard secretion was prepared by adding to a certain number of "dry"

ripe ovarian eggs, double their volume of sea-water. At the end of ten minutes, during which the eggs were slightly agitated at intervals, the suspension was centrifugated, and the eggs cast down. After 100 revolutions the supernatant fluid was carefully decanted and set aside for use.

Ripe eggs were then shaken, usually from the ovaries of a single individual, into a small quantity of fresh sea-water, and to 1 c.c. of a concentrated suspension of these, was added 1 c.c. of the secretion. In this mixture the eggs were allowed to stand 2 hours, when cleavages were usually found in all the dishes. That these were not the result of accidental infection with sperm was guaranteed by special precautions—all dishes, instruments and the hands as well as the animals used having been carefully sterilized. Furthermore, when the first urchin opened was a female, I usually sought no further, as the eggs from a single individual are sufficient in number. Indeed in all but two or three series only eggs from a single urchin were used. It might be urged that the secretion or the sea-water were infected. Sterilization of the latter however does not prevent auto-parthenogenesis, and against the idea that the sperm might have been present in the secretion there are three arguments, two of which are conclusive. In the first place, as has been shown by F. R. Lillie ('13[2]) and myself ('14[2]), the fertilizing power of sperm decreases markedly if they are allowed to remain in the secretion more than a few hours. In some of my experiments the secretion was prepared a day in advance, and used on the eggs of the first urchin opened on the following morning. In the second place fertilization membranes never appeared in any of the experiments with *Arbacia* eggs unless especially induced, and lastly, if the same eggs from which the secretion was prepared were afterwards subjected to its influence for the proper length of time and in the proper concentration, characteristic auto-parthenogenesis took place.

Many experiments were tried varying the concentration of the secretion as well as the time of exposure. My records indicate cleavages at higher concentrations as well as lower, and also in less than two hours, but the greatest number was always obtained when 1 volume of the concentrated egg suspension was exposed

for 2 hours to 1 volume of the standard secretion. If at the end of this time the supernatant fluid is poured off and replaced by fresh sea-water, free-swimming blastulæ will be found within 24 hours. In one case only did development proceed to the pluteus stage.

Among the blastulæ that develop in these cultures are some characterized by an extremely slow rate of movement; others, though rare, may move at a practically normal rate, whereas the majority are considerably below normal in speed. This fact in earlier experiments misled me, as I caught sight of only the fastest swimmers, and mistook the others for arrested cleavages.

The size of the blastulæ is also a ready source of deception, since there are in general three kinds, micro-blastulæ, normal-sized blastulæ, and mega-blastulæ, of which the first are by far the most numerous. As these are usually very transparent and also slow of movement their presence in a culture may easily be overlooked.

The origin of the micro-blastulæ is clear. The first two cleavage cells, although normal in size, in most cases do not remain together, but separate and proceed to develop independently of one another. If the cells produced by these isolated blastomeres continue to separate very minute micro-blastulæ may result. Such falling apart of the cleavage products has been observed in the 16-cell stage and even later. The separation takes place, I believe, because the fertilization membrane has not appeared ('13[1]).

Normal-sized blastulæ need no explanation, since they must result from the eggs whose cleavage cells can be seen to hold together despite the absence of the normal fertilization membrane. As for the mega-blastulæ, it is possible that their origin is identical with that of mega-blastulæ in normal cultures. On the other hand, they were more noticeable in the auto-parthenogenetic series, and it is possible that the larger type of egg is better suited for this than the normal mode of development. Again the possibility is not excluded that the mega-blastulæ may come from eggs of normal size. This is not unlikely, since normal sized blastulæ instantly increase in volume when subjected to the same concentrations of the secretion.

Improved Method of Auto-Parthenogenesis.

Loeb's improved method of artificial parthenogenesis consists in following the treatment with parthenogenetic agents, by an after-treatment with hypertonic sea-water, 8 c.c. of 2.5 *M* NaCl + 50 c.c. sea-water. It seemed likely therefore that a better yield of larvæ could be secured if eggs, after having been subjected to the action of the secretion for two hours, were afterwards treated with the hypertonic solution for forty minutes. This surmise proved correct. The following is an outline of a typical experiment:

Time.	*A*	Time.	*B*
10.20 A.M.	1 c.c. eggs + 1 c.c. secretion.	10.12 A.M.	1 c.c. eggs + 1 c.c. secretion.
12.12 P.M.	Began hypertonic treatment.	1.12 P.M.	Began hypertonic treatment.
12.52	Ended hypertonic treatment.	1.52	Ended hypertonic treatment.
2.30	Considerable number of 2, 3, and 4-cell stages.	2.45	Many cleavages; 2–8 cells. Many normal.
5.45	Numerous 2, 3, and 4-cell stages.	6.00	Many 2, 4, 32, 64 and 128-cell stages.
	Very few 32–64-cell stages.	7.30	Cleavages in every field.
9.30 A.M.	1 active larva, many dead.		Some blastulæ but not free.
		9.45 A.M.	12 fine active larvæ.
			50 per cent. of cleavages arrested.
		4.00 P.M.	Hundreds of slowly moving blastulæ.

In experiment *B* the time of exposure to the secretion was three hours instead of two as in earlier experiments with the hypertonic after-treatment. From this one might infer that better results could have been obtained in the experiments in which the secretion alone was used, but the results do not warrant this assumption. Controls also show that with the hypertonic after-treatment, three hours of exposure to the secretion is better than two. We must believe therefore that the success of experiment *B* and all the others carried out in the same way, depended on the hypertonic after-treatment.

The differences in the length of exposure to the secretion are for the present purely empirical.

In all the experiments both with as well as without the hypertonic after-treatment, development was much delayed in many

of the eggs, and rarely went beyond the blastula stage. As stated before, I observed plutei only once, and not over ten or twelve at that. Gastrulæ also were rare. For this failure to pass beyond the blastula stage there are several reasons; in the first place one must attribute a large share to the fragmentation of the early cleavage stages, due as I believe to the absence of a normal fertilization membrane, and secondly the abnormal permeability relations resulting from the treatment with the secretion probably also play a rôle ('14[2]) by placing mechanical difficulties in the way of gastrulation even though the necessary materials are present in the embryo. The evidence for this conclusion cannot be presented now. Although these obstacles stand in the way of perfecting this method of rearing sea-urchins, the fact that it is impractical, and inferior to other methods does not in the least detract from the theoretical interest of the results. As McClendon ('09) puts it: "In natural parthenogenetic development the end result may be a maturation or segmentation stage or a larva or adult. Though only the reproductive adults are of significance to the species, all are of significance to science."

Auto-Parthenogenesis in *Asterias*.

In earlier papers ('13[1], '14[1] and '14[2]) I have emphasized certain differences as well as certain points of similarity between the eggs of *Arbacia* and *Asterias*. Chief among the differences, are the characteristics of the fertilization membranes; most important among the points of likeness are the decrease in volume to be observed in both kinds of eggs on fertilization, and the fact that the *Asterias* ovum produces a secretion in which the most striking characteristics of the corresponding *Arbacia* exudate can be verified point for point. For these reasons one might anticipate the initiation of development in the *Asterias* egg by the proper employment of its own secretion.

In the following experiments the secretion was prepared exactly as in the case of *Arbacia*, but the eggs before treatment were given time to mature, *i. e.*, they were allowed to stand for about 1 hour in sea-water after removal from the ovaries.

10.27 A.M.	1 volume *Asterias* eggs. 1 volume *Asterias* secretion.	10.27 A.M.	2 volumes *Asterias* eggs. 1 volume *Asterias* secretion.

10.50	Fertilization membranes indicated.	10.53	Fertilization membranes indicated.
12.15 P.M.	Many eggs elongated.		
2.00	Fertilization membranes. Frequent cleavages.	2.00 P.M.	Frequent cleavages.
9.00 A.M.	Cytolysis. One free swimming blastula.	9.00 A.M.	Numerous dead blastulæ. Much gastrulation.

In other tests similar results were obtained. Unfortunately this work came at the end of the *Asterias* breeding season, and further experiments were impossible. Those performed however are sufficient to show that parthenogenesis can be induced in *Asterias* as in *Arbacia* by the use of its own egg-secretion. Owing to the existence of a perivitelline space within which the cleavage products of the *Asterias* egg are held, it appears likely that better results will ultimately be achieved than in the auto-parthenogenesis of *Arbacia*. In fact as indicated above, even without hypertonic after-treatment, which in first experiments was always omitted for the sake of control, one of my auto-parthenogenetic *Asterias* cultures proceeded to the gastrula stage.

"Hetero-Parthenogenesis."

"Hetero-parthenogenesis" as the sequel will show, is probably not the correct word, but I use it here as a convenient heading for experiments in which development was initiated in *Asterias* eggs by the use of *Arbacia* secretion. Such experiments suggested themselves, as the result of the earlier observations on the increase in the permeability of various types of cells. From these one might anticipate "hetero-parthenogenesis," a process which was indeed successfully induced as the following experiment indicates:

10.35 A.M.	1 volume mature *Asterias* eggs.
	1 volume *Arbacia* secretion.
	No immediately visible effect.
10.50	Fertilization membranes indicated.
2.00 P.M.	Numerous cleavages.

The Fertilizin Theory.

After I had discovered auto-parthenogenesis through the observation that the egg-extracts are permeability increasing agents, I consulted with Professor F. R. Lillie who had been performing

those experiments on normal fertilization in *Arbacia* which have led him to propose the fertilizin theory, briefly outlined in his paper "The Mechanism of Fertilization" ('13[3]).

The facts on which this theory is based are dealt with in F. R. Lillie's earlier papers, as well as in the one specifically referred to. In my paper on "A Qualitative Analysis of the Egg-Secretions and Extracts of *Arbacia* and *Asterias*" ('14[2]) will be found certain verifications of Lillie's observations. It would take us too far to go into the factual basis of the theory here, but briefly it postulates as the essential point in fertilization a chain of chemical reactions in which an amboceptor-like substance—the sperm-agglutinating agent—present in the secretions from the egg, unites by means of a spermophile side-chain with receptors in the sperm, and by an ovophile side-chain with receptors in the egg.

If this chain of reactions indeed occurs and is related to fertilization and the initiation of development in the significant manner put forward by Lillie, it follows that there are theoretically at least five possibilities of blocking fertilization. Lillie has listed these as follows:

1. Through loss of fertilizin by the egg.
2. Through occupancy of the sperm receptors.
3. Through occupancy of the egg receptors.
4. Through occupancy of the ovophile side chain of the amboceptor (fertilizin).
5. Through occupancy of the spermophile side-chain of the amboceptor (fertilizin).

Of these Lillie has proved possibility 1, by washing the eggs; 4, by means of an inhibitor in the blood which prevented fertilization, but not the sperm-agglutinin reaction; and 5, by setting free from the egg itself an inhibitor, the anti-fertilizin which obstructs the union of the sperm with the amboceptor by occupying the spermophile side-chain. Since therefore the presence of the agglutinating agent, and moreover its presence in a state in which both spermophile and ovophile side-chains are free to combine with their respective normal receptors in the sperm and the egg, seems to be necessary for normal fertilization, Lillie has called the substance in question, fertilizin.

F. R. Lillie has also offered an interpretation of my results on auto-parthenogenesis. He suggests that in the initiation of development by employment of the egg-secretion, essentially the same chemical chain is involved as in normal fertilization except that the sperm, and naturally the sperm-fertilizin reaction also, is dropped. In other words, in auto-parthenogenesis, the egg-receptors are thought of as combining with the ovophile side-chains of fertilizin which has not been bound to sperm-receptors through its spermophile groups.

Off-hand this supposition does not appear unreasonable. The fertilizin is certainly present in abnormally high concentration in the secretion as used for auto-parthenogenetic initiation, and the mere fact that the secretion is a permeability-increasing agent does not preclude the possibility that one of its constituents may play an additional rôle in the initiation of development. If this is true, it should be possible to block auto-parthenogenesis in accordance with the scheme outlined above, although the absence of the sperm and the sperm-fertilizin reaction restricts the opportunities to 1, the loss of fertilizin; 3, the occupancy of the egg-receptors; and 4, the occupancy of the ovophile side-chain of the fertilizin.

Methods of Preventing Auto-Parthenogenesis.

1. *The Prevention of Auto-parthenogenesis by Washing the Eggs.*—Several preliminary experiments in which the eggs before being exposed to the action of the secretion were washed from 20 minutes to half an hour in great excesses of sea-water, proved conclusively that this procedure very materially reduces the number of cleavages, and the number of larvæ, afterwards found in the cultures. These results led to a more careful test carried out on eggs which had been washed in excesses of sea-water for 24 hours. The sea-water was changed frequently but at irregular intervals. These eggs were then treated in the usual way with the secretion, but not a single cleavage was found. Thus the effect of even slight washing is noticeable whereas prolonged washing effectually prevents the auto-parthenogenetic cleavage. From this we might conclude that something essential for the initiation of development had been removed from the eggs.

However the failure of the fertilizin with which the eggs were treated and for which they are permeable, to offset the effects of washing, remains to be accounted for.

2. *The Prevention of Auto-parthenogenesis by Means of a Group Split from the Sperm.*—Lillie has made the apparently justifiable assumption that fertilizin bound by sperm is activated, *i. e.*, has a greater chemical affinity for the substances with which it unites in the egg than the unbound fertilizin. If this is true, it occurred to me that the use of sperm, in conjunction with the secretion might give better auto-parthenogenetic results than had been obtained by the use of the secretion alone, either with or without the hypertonic after-treatment. Naturally only dead or seriously injured sperm are available for this experiment, and with the latter one could not be sure to exclude a certain and misleading number of real fertilizations which would then be mistaken for very fine cases of parthenogenesis. Since the power of the fertilizin to produce the agglutination reaction and to play a part in normal fertilization is not destroyed by brief boiling, it seemed possible that the complementary chemical groups in the sperm might also survive boiling without being destroyed or so altered as to be incapable of taking some part at least in the reactions between egg and fertilizin.

However, instead of furthering auto-parthenogenesis, exactly the opposite effect was found, for the boiled sperm absolutely prevented the process, as shown by the following experiment:

1 volume of eggs+1 volume of secretion[1]+2 volumes boiled sperm infusion 10.40 A.M. to 2.50 P.M. Four hours later little if any effect visible; a few cell-masses were noted, but nothing that in any way suggested auto-parthenogenesis. At 2.58 P.M. the mixture was diluted with an excess of sea-water. At 6 P.M. no effect was noticeable; by 10 A.M. the next day, the eggs had undergone a granular decomposition.

Experiment repeated carefully controlled. Result identical. In the control however auto-parthenogenesis occurred and on the following morning 12 very fine larvæ were seen in addition to the usual number of delayed cleavages and micro-blastulæ.

[1] Unfortunately the egg-secretion in these tests was more dilute than usual, but the effect noted is not interpretable on this basis since parthenogenesis occurs at greater dilutions. The sequel to these experiments further emphasizes the interpretation given here.

Since the fertilizin theory postulates essentially the same machinery for auto-parthenogenesis and normal fertilization it follows that initiation by living sperm should also be blocked by treating the eggs with the boiled sperm-infusion. This is actually the case:

Eggs treated for two hours with strong boiled sperm-infusion. At the end of this period they were heavily inseminated with living sperm, but remained unfertilized. Only a few cleavages were observed later, but these were arrested at the latest in the four-cell stage. No further development occurred, but on the following day practically all the eggs exhibited fertilization membranes. Controls normal.

3. *The Prevention of Auto-parthenogenesis by Means of a Group Split from the Secretion.*—F. R. Lillie ('13[2]) finds the agglutinative power of egg-secretion unimpaired, or perhaps better, not measurably impaired, by boiling for 5 minutes, although the solution undergoes a change of color due to the formation of small quantities of a purple compound ('14[2]). As the secretion is thus visibly different in chemical composition it becomes important to ascertain whether the formation or presence of the purple compound makes any recognizable difference in the initiation of development. The following experiments indicate that the efficacy of the secretion as a parthenogenetic agent is blocked.

Standard secretion boiled. Purple compound formed. Sperm-agglutination positive.

3.20 P.M. 1 volume of boiled secretion added to 1 volume of fresh egg-suspension.

8.00 P.M. No cleavages.

8.00 A.M. Few delayed cleavages, not at all in normal frequency.

11.30 A.M. 1 volume of boiled secretion added to 1 volume of fresh egg-suspension.

2.00 P. M. No cleavages.

7.00 P.M. No cleavages.

8.30 A.M. No cleavages.

Control in unboiled secretion exhibited auto-parthenogenesis in usual quantity.

Interpretation of Experiments on Prevention of Auto-Parthenogenesis.

Analysis of the means by which a process can be prevented may be even more suggestive than a similar analysis of the means by which it can be induced, and this proves to be true in the present case. How then are these experiments to be understood?

1. *The Block in Washed Eggs.*—The prevention of fertilization by washing (F. R. Lillie, '13[3]), according to the fertilizin theory, is due to the absence of the intermediary body with which both sperm and egg receptors normally unite. We cannot, however, despite the superficial similarity of the two cases, attribute the auto-parthenogenetic block to the absence of suitable bonds for the egg receptors since the surface film of the ovum is perfectly permeable for fertilizin. This must diffuse into the eggs from which it has been removed the moment they are exposed to the concentrations employed for initiation. If this is correct, then the prevention of auto-parthenogenesis in washed eggs must be explained in some other way. This however is not necessarily fatal to the fertilizin theory, for these same experiments strongly suggest that a mere increase in permeability is insufficient to initiate development, a suggestion still further reinforced by the second type of block to be considered presently.

The experiments with washed eggs are also suggestive from another standpoint. Lillie (*loc. cit.*) tells us: "If it be true that the egg contains its own fertilizing substance it might also be possible to induce parthenogenesis by increasing the concentration of this substance to a certain point. . . ." The washed eggs however demonstrate that the fertilizin experimentally added to the solution about the eggs has no fertilizing effect, and if it has none when the eggs have been washed, there is no good ground for considering its effect different when the eggs have not been washed. "Hetero-parthenogenesis" strongly backs up this position. It appears most likely therefore that in auto-parthenogenesis the fertilizin experimentally added, plays the same rôle as any other permeability increasing agent effective in the artificial initiation of development. If this is correct, and furthermore, if the parthenogenetic block in washed eggs is not identical with the fertilization block brought about by the same means,

it follows, not that the fertilizin theory is inapplicable to these cases, but only that it does not apply exactly as F. R. Lillie has supposed.

2. *The Block by Means of Groups Split from the Sperm and the Egg-Secretion.*—The prevention of both fertilization and auto-parthenogenesis in eggs previously treated with boiled sperm infusion, as well as the parthenogenetic block in eggs subjected to the action of boiled secretion, seem at first sight to have little in common. In reality however there is an element of identity in all three cases, for suspensions of sperm as well as the egg-secretion give on boiling a characteristic color reaction due to the formation of a purple substance which may be looked upon as a chemical cleavage product split off from a mother substance. In an earlier paper ('14[2]) I suggested on other grounds, and in complete ignorance of its rôle in parthenogenesis, that this purple material might prove to be significant, as it seems to be specific for *Arbacia*. This suggestion now appears almost certainly correct, and I therefore propose for this substance, provisionally at least, the name "Purple X."

An attempt to understand the part played by Purple X in these reactions must begin with the proof that this substance and not some other cleavage product is really the effective one. Such proof would be found if the block to both parthenogenesis and fertilization could be removed with the removal of the Purple X. To accomplish this and at the same time not render the solutions toxic by the addition of precipitating agents, called for methods which had first to be discovered.

The Purple X is either in a state of very fine suspension, bordering on solution, or is actually in solution. Since now a precipitable colloid frequently carries other bodies with it when thrown down, I added the albumen of hen's egg to the *Arbacia* egg-secretion, and boiled the mixture. The expectation that the purple compound might be removed at least to a considerable extent, proved correct, as the white coagulum of hen's egg albumen was quite markedly discolored by the purple substance. After filtration the secretion was tested for its parthenogenetic effect. The method of separation however is not quantitative and so the results were not perfectly clear cut. Nevertheless it

remains true that the only boiled secretion that ever induced parthenogenesis was one from which the Purple X had been removed by coagulating white of hen's egg. This result, I may add parenthetically was obtained only after a very remarkable effect of the egg-white itself had been investigated. Even in the dilutions in which it is present in the sea-water after boiling, the white of a hen's egg produces very regular and curious distortions of the eggs which may even be divided into pseudo-cleavage products. However the effect does not seem to be analogous to parthenogenesis, and I shall therefore deal with it separately at another time.

In the course of these experiments a very much better and simpler method was found, for the Purple X is a relatively unstable compound and readily disappears. Thus a concentrated sperm-suspension boiled on one day and giving a color as deep as port wine, may on the next be golden yellow. With such a solution eggs were treated for one hour and five minutes. At the end of that time the addition of living sperm resulted in the fertilization of practically every egg. The experiment was repeated, the eggs being exposed for two hours and forty minutes, and again fertilization occurred.

As a counterpart to these experiments with sperm, the parthenogenetic effect of boiled secretion which had lost its purple color was tried. Such solutions contrast sharply with the newly boiled secretion, for whereas the latter very effectually blocks auto-parthenogenesis, development can be induced without difficulty after the spontaneous disappearance of the Purple X.

Since the Purple X-block occurs in the presence as well as in the absence of sperm, we can be certain that it is not the result of an effect upon the male sex cells or, in the language of the fertilizin theory, of occupancy of the sperm receptors. Which particular one of the remaining three blocks theoretically possible we are dealing with however must for the present remain an open question, although if the immediate effect of the egg-secretion is really identical with the immediate effect of any other parthenogenetic agent, occupancy of the egg-receptors is indicated. If this is true, then Purple X should block parthenogenesis no matter how induced. That this process should be blocked by

less than is required to prevent fertilization is perfectly intelligible on Lillie's assumption that sperm-bound fertilizin is activated and has a greater affinity for the egg-receptors than free fertilizin.[1]

An Attempt to Construct a Provisional Working Hypothesis.

Although no final interpretation of the phenomena of fertilization is possible at the present time, an attempt to formulate some viewpoint which does no violence to our knowledge, and which at the same time may serve as a working hypothesis, must be made for further guidance.

If we accept the evidence of F. R. Lillie, together with my verifications and additions to it, and remember that many of the facts discovered by Lillie, as well as some found by myself, are the direct products of the fertilizin theory considered as a tool, I think we may even now admit that it is a useful working hypothesis. Lillie ('13) himself has emphasized this point, and moreover has listed among the other advantages of his theory, that it gives us an explanation of the specificities of fertilization; may furnish the foundations for the chemical conceptions needed by any theory of fertilization, and above all, that it offers one explanation for the initiation of development, whether by fertilization or parthenogenesis.

To what extent these claims are justified, and to what extent the theory will undergo modification, it would be unprofitable to discuss at present. One claim and its consequences however does invite discussion at the present time, and in this connection, for if the fertilizin theory is really capable of unifying the various methods of inducing parthenogenesis, its relations to another view, which apparently does the same thing must be analyzed, and furthermore we must answer the question whether all cases of parthenogenesis are really instances of auto-parthenogenesis.

[1] Since Purple X also appears when spermatozoa come into contact with the secretion, it is possible that its appearance plays a rôle in the prevention of polyspermy. This would not prevent a similar effect on the part of the anti-fertilizin, as suggested by F. R. Lillie. Indeed the chances that any particular sperm will complete the reactions postulated by the theory, must be exceedingly small when we reflect on the immense numbers which collect about each egg. Such collections become more intelligible when we recall that whereas the biparental effect can be carried out by a single sperm, the initiatory effect, at least in *Arbacia* ('13[1]) requires more than five.

1. *The Surface-Alteration Theory.*—The view which we may call the surface-alteration theory is a product of the work of Loeb, R. S. Lillie, and many others. According to Loeb the initiation of development depends in the first place upon a superficial cytolysis, or destruction of the cortical layer of the egg, brought about either by a lysin in the sperm, or by the so-called parthenogenetic methods and agents. After this alteration of the surface the rate of oxidation in the egg is raised from four to six times. Loeb says:

"There are two possibilities by which this result can be produced: either a catalyzer (an oxidase) is carried into the egg by the spermatozoon; or the change in the surface layer itself causes the increased rate of oxidation. Everything speaks in favor of the second assumption."[1] Further on Loeb suggests "that the spermatozoon causes development . . . by removing an obstacle to development."[2]

R. S. Lillie ('11 and earlier papers) has suggested that the superficial cytolysis increases the permeability of the egg, and further that through this increase in permeability a substance antagonistic to oxidation is eliminated from the egg. This substance he imagined might be CO_2 ('09).

Against this idea Loeb has raised objections. Inasmuch as CO_2 "is a good agency for calling forth membrane formation" and as only substances capable of diffusing into the egg can have this effect, the egg surface must be permeable to CO_2 before fertilization, and consequently there can be no accumulation that could be relieved as the result of an increase in permeability.

This criticism seems valid, and while it constitutes a reason for giving up the idea that the antagonist lost is CO_2, it in no way bears on the other idea, namely that there is an increase in the permeability of the ovum as the result of fertilization. However this idea also has failed of acceptance by Loeb, but this is due

[1] "Artificial Parthenogenesis and Fertilization," 1913, p. 13.

[2] The obstacle "removed" is a cortical layer unsuited for development, and the "removal" is in reality an alteration of this cortex: "Through cytolysis of the cortical layer of the egg the oxidations in the unfertilized egg are accelerated from four to six times their normal rate" (loc. cit., p. 14). Since this heightened oxidation persists after fertilization, and since the permeability of fertilized eggs may not be different from that of unfertilized, it is possible that the cytolysis of the cortical layer alone is insufficient to account for the increase in the rate of oxidation.

chiefly I believe because the evidence in support of it has been unfortunate not only in a purely technical sense which has been justly criticized, but also because it was gathered under a misapprehension apparently as to exactly what R. S. Lillie had suggested. So far as I can see, experiments in which the permeability of fertilized eggs is compared with that of unfertilized have no bearing on the question, for no such difference was postulated, but only that the "increased permeability is . . . temporary in normal or in favorable parthenogenetic fertilization" ('11, p. 307).

If this idea is correct we might expect two things to happen at fertilization in the egg of *Arbacia*. Since this egg before impregnation actively secretes materials that discolor the sea-water, the discoloration produced by eggs undergoing fertilization might be greater. I have found ('14[2]) that it is one and a half times that of eggs not undergoing impregnation. Further, fertilized eggs, after the process is complete, do not discolor the sea-water. In the second place even a temporary increase in permeability should result in a change in volume if the eggs remain in a medium that remains constant during the period under discussion. Measurements show ('14[1]) that the eggs of both *Arbacia* and *Asterias* are smaller in volume after fertilization than before.

I therefore consider the idea of increased permeability *during* fertilization correct, and that we may suppose all cases of fertilization, whether with sperm or without to have this one point in common. Since many of the results now published had not been found at the time I wrote my preliminary paper ('13[1]) I threw emphasis on this common attribute in section VIII. This emphasis I do not wish to withdraw as the result of later work, for this has only strengthened my conviction that in fertilization an increase in the permeability of the ovum actually occurs. The only question at issue seems to me to be the significance of this change for the initiation of development.

On this particular point I also postulated the loss of substances antagonistic to oxidation. This suggestion was based on the fact that substances are lost during the act of fertilization at a higher rate than before, and finally that the presence of egg-secretion in certain concentrations retards development. The

retardation I supposed to be due to the depression in the rate of oxidation. This assumption, as I pointed out, does not seem unreasonable; on the other hand as I pointed out more recently, retardation of development may in this case also be due to interference with, or depression of, other processes no less essential. Only further work can decide these questions.

2. *The Fertilizin Theory.*—In the fertilizin theory, no particular rôle is assigned to the increased permeability. "The nature of the effect of the activated fertilizin on the egg is analogous in some respects to a superficial cytolysis, in this respect agreeing with Loeb's theory. But the 'lysin'[1] is contained in the egg, not in the sperm as Loeb thought; if cytolysis is involved, it is a case of auto-cytolysis. This may involve increase of permeability, the effects of which R. S. Lillie especially studied" ('13[3]). These possibilities were mentioned "in order to point out that the conception" of the fertilizin theory "is not in conflict with the well-established work of others." This is very important for the fertilizin hypothesis is based on certain facts which seem to permit the interpretation given them, and the surface-alteration theory also has a verifiable factual basis. These facts, occurrences in and about fertilization, cannot be in conflict with one another. The problem is to understand their interrelations. The experiments on auto-parthenogenesis carry us a step forward in this direction.

At first sight success in initiating the development of *Asterias* eggs by means of *Arbacia* egg-secretion would appear to seal the death-warrant of Lillie's fertilizin theory. On the other hand a little reflection will show that no radical conclusions need be drawn. Parthenogenesis, as is well known can be induced in different eggs by the most diverse means—electricity, heat, cold, mechanical shock, osmotic pressure,[2] specific chemical alteration

[1] It would have been more correct to say: it *was* contained in the egg.

[2] In the case of the purely osmotic methods, Loeb supposes that "the hypertonic solution acts simultaneously in two capacities: first as a cytolytic agent causing a change in the cortical layer (the formation of a gelatinous film) and second, as a corrective agency" ('13[4], p. 159).

The cytolytic action of the hypertonic solution is not without evidence, and so we may consider this method as having the effect common to all the others. If however the increase in permeability is effective also on account of the destruction of the normal fertilization block, in the sense outlined in the text, loss of water by the egg would directly or indirectly accelerate the loss of the antagonists (cp. Glaser, '13, p. 450).

of the surface film or cortical layer, lipoid solvents, and even the prick of a needle having been found effective. These methods, each of which is capable of variation and modification, have nothing in common except their result, and it is idle to try to imagine any direct causal connection between them and the result. However one effect, common to all, seems to stand intermediate between the parthenogenetic result, and the experimental means used to induce it. This common intermediary is a change in the permeability of the surface of the eggs, and in the experiment in which parthenogenesis is induced in one ovum by the secretion of another it is not necessary to assume that anything occurs that does not also take place in all the other cases. The same reasoning applies also to auto-parthenogenesis, a process which was found by application of the surface-alteration theory, and in total ignorance of the fertilizin hypothesis.

Now the picture of fertilization with sperm given by the fertilizin theory is briefly the sperm-receptor-amboceptor-egg-receptor reaction. In the picture of auto-parthenogenesis, the sperm is dropped, leaving the amboceptor-egg-receptor to react, the former with an unsatisfied bond. Since however parthenogenesis can be induced without the addition of fertilizin to the eggs there is no reason for supposing that the egg-receptors united with the fertilizin which was added in my experiments for the purposes of initiation. This however does not seem to me to be at all necessary, for both substances are present in the egg from the beginning and we may postulate a union between the receptors and the fertilizin within the egg.

If this is indeed the mechanism of both parthenogenesis and auto-parthenogenesis, we must answer the question why the egg was not fertilized before, or what amounts to the same thing, why fertilization occurs at all.

In speaking of parthenogenetic agents, Lillie (*loc. cit.*) tells us, they "need only remove obstacles to the union of the amboceptor and the egg-receptor" (p. 527); and in connection with auto-parthenogenesis, that this might result "if the concentration of fertilizin were raised to a certain point, though it is conceivable that no increase in concentration would break down the resistance that normally exists to the union of the amboceptors and egg-

receptors" (527). In other words, the egg is not always fertilized, because there are obstacles or resistances. When these are removed, fertilization, parthenogenesis, or auto-parthenogenesis may occur.

No circumstance connected with the initiation of development that is not a common occurrence in all cases is capable of explaining how these obstacles or resistances can be removed. It is not necessary for clear thinking on this subject to know what they are at present, but the only common fact which seems capable of explaining how these natural blocks to fertilization, and the initiation of development could be removed, is at present the increase in the permeability of the ovum. For this reason, without in any way trying to minimize the significance of the facts emphasized by the fertilizin theory, I believe that the permeability change plays a very real part in the process of fertilization, for through it is possible a disturbance of the equilibrium in which we know the egg finds itself prior to impregnation or parthenogenetic initiation.

If this viewpoint is correct, the following picture of both fertilization and parthenogenesis is possible. Fertilizin alone in sufficient concentrations is capable of increasing the permeability of the ovum; smaller quantities when activated by union with sperm are able to exert the same effect. This alteration of the surface may also be brought about by electricity, heat, cold, mechanical shock, specific chemical alteration of the membrane, lipoid solvents, pricking, or any other way of rendering the egg surface more permeable or of accomplishing the same result as an increase in permeability. The immediate effect of this permeability increase is a removal of those obstacles or resistances which constitute the normal fertilization block. These removed, the fertilizin within the egg combines with the egg-receptors, but if fertilizin activated by sperm is present, the egg receptors would combine with the elements for which ex hypothese they have the greater affinity.

So far the facts seem to group themselves harmoniously, but how are we to conceive of the "obstacles" or "resistances"? We might imagine the existence within the unfertilized egg of a body capable of playing the required rôle, but for this assumption

there is no evidence. Moreover the substances demonstrated by F. R. Lillie, seem to me sufficient to account for the normal fertilization block if we make a much simpler assumption—one indeed which appears necessary on chemical grounds.

The ovum, according to Lillie, contains egg-receptors, fertilizin, and antifertilizin. Chemical affinities exist between the anti-fertilizin and the spermophile groups as well as between the receptors and the ovophile groups. Although Lillie does not postulate a reaction involving these elements in the unfertilized egg, it is difficult to believe that three substances, demonstrably capable of uniting in the manner indicated, would lie side by side without entering into combination, however loose. If this assumption prove valid, we can consider this union as constituting the normal fertilization block ("resistance," "obstacle") of the unfertilized egg.

From this standpoint it appears possible to explain the block to parthenogenesis in washed eggs. The egg surface prior to fertilization or treatment with parthenogenetic agents, is perfectly permeable to fertilizin. Lillie has shown, however, that the anti-fertilizin can only be removed by destruction of the ova, whose surfaces, with respect to these two substances, appear to act like dialysers. It is quite likely therefore that washed eggs differ from unwashed by a relatively higher concentration of anti-fertilizin. If now fertilizin is permitted to diffuse into a washed egg, it would be bound by the anti-fertilizin and so an initiatory effect, due to its further union (if indeed this occurs[1]) with the egg-receptors would be prevented. According to Lillie's view and the law of mass action, auto-parthenogenesis might occur in washed eggs when the concentration of fertilizin is more than enough to bind the anti-fertilizin. That it takes about two hours to initiate auto-parthenogenesis, and that I failed to initiate it in that length of time in washed ova, are both in harmony with the above suggestion.

So far as I can see, this is the only way at present, in which it seems possible to draw a picture in which all the facts and all the cases may find a place. It follows from the preceding reason-

[1] The avidity of fertilizin bound by sperm for the egg-receptors is assumed by Lillie to be greater than in unbound fertilizin. The opposite effect, resulting from a union of the spermophile groups with the anti-fertilizin is not excluded.

ing, as well as more directly from the experiments themselves, that the rôle of the egg secretion (fertilizin) experimentally added to induce development was exactly the same as the rôle of any other parthenogenetic agent, and that it united with the egg receptors, no more than do the electricity, heat, cold, mechanical shock, osmotic pressure, or what-not. On the other hand, like these, the egg-secretion by affecting permeability, brings about conditions under which the ovum is freed from its inhibitors and, in a sense, enabled to fertilize itself. From this standpoint all parthenogenetic methods are methods for inducing auto-parthenogenesis, and fertilization with sperm differs from auto-initiation simply in the fact that the fertilizin which unites with the egg receptors has itself previously united with the sperm in the jelly, or the water about the egg.[1]

Summary.

1. The egg-extracts as well as the egg-secretions of *Arbacia* and *Asterias*, in addition to characteristic effects on spermatozoa, are permeability increasing agents, and initiate development. For the type of initiation here dealt with I have chosen the term *auto-parthenogenesis*.

2. Auto-parthenogenesis is blocked in *Arbacia* by washing the eggs in sea-water.

3. A group "Purple X" which may be split off from the sperm of *Arbacia*, and which also appears in boiled egg-secretion, blocks parthenogenesis in low concentrations and fertilization in high concentrations.

4. Prevention of parthenogenesis by washing can hardly be the result of an absence of fertilizin; prevention by Purple X may be the result of occupancy of the egg-receptors.

5. It is not unlikely that all cases of parthenogenesis are in reality instances of auto-parthenogenesis.

6. With certain minor modifications it appears possible to

[1] According to F. R. Lillie's account, the sperm unites with the fertilizin in the egg. This however seems incredible, for the affinity between sperm and fertilizin is very great, and since the egg jelly, through which the sperm must pass on their way to the ovum is heavily charged with the fertilizin, it is difficult to see how any unbound sperm could reach the egg. This change in the geographical location of the sperm-fertilizin reaction however makes absolutely no difference in essentials.

construct an hypothesis of fertilization in which the basal facts of the surface-alteration theory, as well as the facts emphasized by the fertilizin theory may, without violence to our knowledge, find a place. From the standpoint here adopted, there is no antagonism between the two theories which emphasize different groups of facts associated with the process of fertilization. These facts cannot be in conflict with one another.

UNIVERSITY OF MICHIGAN,
January 10, 1914.

LITERATURE.

Loeb, Jacques.

'11 On the Fertilizing Effect of Foreign Blood Serum upon the Egg of the Sea-Urchin. Proceed. Path. Soc. Phila., 1911.

Lillie, R. S.

'11 The Physiology of Cell-Division. Amer. Journ. Physiol., XXVII.

McClendon, J. F.

'09 On Artificial Parthenogenesis of the Sea-Urchin Egg. Science, XXX.

Lillie, F. R.

'12 The Production of Iso-Agglutinins by Ova. Science, XXXVI.

Glaser, Otto.

'13[1] On Inducing Development in the Sea-Urchin (Arbacia punctulata) together with Considerations on the Initiatory Effect of Fertilization. Science, XXXVIII.

Lillie, F. R.

'13[2] Studies of Fertilization, V. Journ. Exp. Zool., 14.

'13[3] The Mechanism of Fertilization. Science, XXXVIII.

Loeb, Jacques.

'13[4] Artificial Parthenogenesis and Fertilization. Chicago, 1913.

Glaser, Otto.

'14[1] The Change in Volume of Arbacia and Asterias Eggs at Fertilization. BIOL. BULL., Vol. XXVI.

'14[2] A Qualitative Analysis of the Egg-Secretions and Extracts of Arbacia and Asterias. BIOL.. BULL, Vol. XXVI.